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AN ANALYSIS OF THE NERVOUS CONTROL OF THE CARDIOVASCULAR CHANGES DURING OCCLUSION OF THE HEAD ARTERIES IN CATS

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STATEMENT OF THE PROBLEM.[1] The relations dealt with in this study are the cardio-vascular relations found in the mammalian organism under extreme conditions of stress. The procedure of the experiments, occlusion of the head arteries, gives a complete anemia of the brain, and thus produces a profound change in the internal environment of the animal. To this the mammal tends to respond by a series of vigorous reactions. These reactions, moreover, seem to go in a direction opposite to that of the change in internal conditions of a particular group of cells. Thus, with an asphyxial accumulation of carbon dioxide in the medium surrounding the critical medullary cells, there is released an entire series of reactions which, could they all be carried to completion, would reduce the tension of this gas in the body fluids of the cerebral region. Prominent among these reactions is a great and prolonged rise of blood pressure, involving the extreme resources of the organism, tending to send a greater volume of blood to the anemic regions, and hence to decrease the concentration of the carbon dioxide in the nerve cells of the medulla oblongata. In the cat, this anemic rise of blood pressure can be well controlled anatomically, and is suscep-

[1] A preliminary note has been published in Proc. Soc. Exper. Biol. and Med., 1921, xviii, 155.

tible of rather exact registration. Moreover, artificial respiration may be maintained throughout the reaction, and thus the activity of the peripheral mechanisms, the heart, blood vessels and internal secretions, be kept free from the central asphyxial changes. Furthermore, under artificial respiration, the reaction may be obtained repeatedly in the same animal. It has therefore offered an opportunity for analyzing the factors involved in such an emergency reaction to inimical conditions in the central mechanism.

Since the work of Ludwig, Cyon and Bezold in the sixties, the importance of the splanchnic vasomotor fibers for the production of extensive changes in blood pressure has been recognized. The related action of the discharge of adrenalin into the blood stream has recently received considerable emphasis. However, the degree to which either the splanchnic constrictor fibers or the secretion of the adrenal glands is involved under such conditions of stress as evoke the anemic rise, has not been evaluated with sufficient accuracy. This study has therefore been concerned particularly with the efferent nervous pathways of the "anemic rise" of pressure: above all, with the degree to which it involves the splanchnic constrictor fibers. The extent to which splanchnic involvement has made for adrenal activity has then been investigated. Finally, the influence of the cardiac innervation, insofar as this may directly effect changes in the level of blood pressure during anemia, has also been examined.

Through the restriction of the effect of the arterial occlusion to the head region alone, the activation of the vascular response by the medulla oblongata is under close experimental control. Accordingly, the central relations of the various nervous levels controlling the efferent channels could also be investigated. Indeed, the analysis of the peripheral factors was in large part undertaken in order to establish more accurately the functional organization of the central nervous mechanism upon which the vascular response depends, that is, the extent to which the peripheral agents executing the vascular responses of the intact animal are activated either by the higher nervous levels, or by the spinal cord alone.

This analysis was undertaken in connection with the studies on the central nervous system carried out under Prof. F. H. Pike, which have dealt particularly with the bearing of its organization on the problem of "spinal shock." In connection with the problems here opened up it was necessary to ascertain the exact nature of the peripheral and central factors controlling the typical vascular response in animals in

which either no lesions within the central system were undertaken, or when these were inflicted, no interval for recovery after the operation was allowed. In comparison with such data, a study of the vascular responses after recovery from transection of the spinal cord could be undertaken more intelligently, and the actual changes wrought by the so-called shock effect evaluated with greater precision.

HISTORY OF THE METHOD. Initiated by the very early work on abdominal ligation of Stenson (1) and Swammerdam (2), Magendie and Poiseuille (3) and Sir Astley Cooper (4) in the early nineteenth century worked out the procedure of cerebral ligation, particularly the isolation of the four chief arterial channels to the head, and noted the circulatory changes which followed. Batelli (5) and Hill (6) have given the earlier history of the procedure in some detail.

The experiments of the eighteen fifties and sixties led to the recognition of the nervous organs as the chief agents in activating the changes following arterial ligation: thus the work of Kussmaul and Tenner (7), Brown Sequard (8), (9) and Vulpian (10) on the head area and of Schiffer (11) on the spinal cord. The emphasis of the importance of the medulla for the maintenance of life as given by the work of Le Gallois and its extension by Flourens (12) was still more increased by the localization in the same region of the vasoconstrictor center as soon accomplished by Ludwig (13), Owsjannikow and Dittmar, and soon led up to the most complete studies on occlusion of the head arteries carried out by Sigmund Mayer (14), (15). Mayer not only described the series of changes following anemia with great detail and accuracy, but also recognized that the elicitation of the anemic rise was dependent on conditions of functional conductivity within the brain stem. He also saw that occlusion of the head arteries was comparable to decapitation with the knife, and that the various functions retained following cerebral ligation were all to be attributed to the activity of the spinal cord, notably the residual spinal level of blood pressure of 50 to 60 mm.

Couty (16) produced circulatory obstruction in the head region by the injection of lycopodium spores. This work, contemporaneous with that of Mayer and equally detailed, but carried out under the influence of the earlier work of Goltz (17), (18) and Vulpian, stressed the residual spinal functions, maintained following isolation of the medulla. Subsequent work on cerebral anemia was almost exclusively done from this point of view. Thus the papers of Schlesinger (20), Kowalewsky and Adamük (21), Bochefontaine and Vulpian (22), Mayer (23), attempted to combat this viewpoint by an analysis of the differential

effect of compression of the abdominal aorta. Konow and Stenbeck (24) and Landergren (25) more recently stressed the functional survival of the cord in the decapitated animal preparation. The residual spinal blood pressure was analyzed by Pike (26) (1912) who showed that afferent impulses, presumably from skeletal muscles, were responsible for it. His observation that a further fall occurs on paralysis of skeletal muscles by curare has recently been confirmed by Langley, 1919 (27).

A revival of interest in the central relations of the asphyxial picture, particularly to the higher nervous levels, was in part achieved through the reëxamination of the problems of resuscitation of the organism by Stewart, (28), (29), (30), (31), (32), (33) Pike and Guthrie. These observations threw sharply into relief the dependence of resuscitation on the medullary respiratory and vasoconstrictor mechanisms rather than on other organs, which, whatever their importance, were found neither as sensitive nor as susceptible as the medullary and higher cells. The functional activity of the medulla was abolished 15 minutes or more, and in its abeyance, no independent existence of the animal could be reëstablished. An analysis of the conditions of so-called spinal shock was undertaken by Pike (34), (35), (26), who employed the procedure of cerebral anemia, and the vascular response obtainable from it, as a means of comparing the various functional levels of the central nervous system. In this way the central relations, particularly to the bulbar levels, of the vascular response in anemia were clearly indicated. A further extension of this problem is found in the study of Yates (36), in which the response to anemia was used as a criterion of the degree of recovery of the vascular system following spinal transection. These studies bring out the importance of the maintenance of medullary activity as the essential factor in the avoidance of a shock effect and the relative incompetence of the spinal cord in the initiation of significant adaptive responses.

Consideration of the excitatory and depressing effects of the blood gases has led toward a recognition of their importance in influencing the behavior of the medullary cells. The literature of the subject is reviewed by Bethe (37), Hill and Flack (38), Hasselbach (39). Pike, Coombs and Hastings (40), (41) have pointed out the adaptive nature of the nervous changes induced by a rapid lowering of CO_2 tension in dyspneic blood, and have suggested that in thus acting in a direction opposite to environmental change, the organism meets the conditions by adjustment of physical equilibrium as prescribed by le Chatelier's

theorem. Mathison (42), (43) has shown the very much greater sensitivity of the medullary over the spinal cells in their response to the asphyxial agents such as increased CO_2 or lactic acid, or decreased oxygen. Pike and Scott (44) have discussed the regulation of H-ion concentration in connection with the regulation of mammalian internal environment.

METHOD. In the present study advantage was taken of the reversibility of the procedure of cerebral anemia. The ability to repeat the initial stimulating effect of the insult on the medullary cells was exploited, rather than its abolition of conductivity within them. The specific problems attacked were dealt with in terms of the intensity and duration of the anemic rise, under given central and peripheral lesions. A seemingly significant series of observations on the changes at the periphery could be obtained by means of the pronounced differences in the character of the curves recorded.

Mayer (14) had called attention to the fact that the magnitude of the vasomotor effect under asphyxia could be approached only by the effect of compression of the thoracic aorta, or injection of strychnine. From Mathison's work (42), (43), especially from his conception that all forms of asphyxia are due to definite increase of the acid content of the blood, cerebral anemia can probably be assumed always to be acting at a maximum. The procedure followed was essentially that indicated by Stewart (28). As here used, the emphasis lay especially on the restriction of the occlusion time to as narrow a limit as possible, in order to insure more rapid recovery. Accordingly, the shortest possible occlusion period was uniformly employed and as a routine procedure the head arteries were released as soon as the spontaneous fall of pressure at the end of the response set in.

The experiments were all carried out on cats. Ether was the anesthetic uniformly employed, and administered by tracheal cannula. The purpose of the study was essentially to determine the degree of involvement of the chief factors concerned, rather than their minute evaluation. This has been left for subsequent study. The extensive series of Stewart served as a basis of comparison and control.

The head arteries were all secured outside the thoracic wall, the branches of the left subclavian, separately secured in the axilla, the right carotid, and right subclavian from within the carotid sheath in the neck; the left carotid held the blood pressure cannula. All the arteries were kept under ligatures ready to be occluded by clamps at the convenience of the experimenter. Since there was no interference

with extra-pulmonic pressure through the operative procedure, artificial respiration could be dispensed with as long as the medullary cells remained functional.

Prior to occlusion, ether was reduced until various obvious tests of the activity of the brain stem could be secured, the return of a vigorous corneal reflex always being awaited before the circulatory arrest was made. With the elicitation of the corneal reflex, artificial respiration was begun, and the clamps on the arteries immediately adjusted. Care is needed to include all the arterial branches isolated in the clamps.

With the adjustment of the clamps, the entire series of peripheral effects follows; the eye reflexes are immediately lost, and within about 20 seconds the more marked peripheral effects are released. Deep and labored breathing sets in, skeletal convulsions appear, and a sharp rise of blood pressure is recorded which often reaches 200 mm. Hg. or more (fig. 5a). This frequently outlasts the other functions; the pressure may not begin to fall until some 10 to 80 seconds after respiratory failure.

The time from the shutting off of the arteries to the circulatory failure is then taken as the complete occlusion time. On the average, this occupied 3 minutes.

Immediately following reëstablishment of the circulation there is a profound depression of all functions. Blood pressure continues falling markedly when the arteries are released, and finally reaches a level of about 50 mm. No other medullary responses are elicitable at this time. Artificial respiration is, of course, maintained throughout the period of depression, and until such time as the bulbar functions again become evident.

If no further lesions are inflicted, occlusions of 3 to 4 minutes are usually followed by a beginning of recovery within 5 to 7 minutes after release of the arteries. Blood pressure usually starts rising first, and after a rise of 10 to 15 mm. spontaneous respiratory gasps reappear. Pressure continues to rise, respiratory movements become more and more frequent; soon normal pressure is regained and the animal breathes quietly and regularly. Ten to 15 minutes after release of the arteries, pressure is usually normal, vibrissae are erect, and the corneal reflex is again elicitable. At this point, a renewed occlusion of the head arteries may be done and the entire cycle repeated.

The modification of anatomical conditions was usually carried out in the interval of depression following a control occlusion. In this way further etherization was avoided. Except under certain specified

conditions, the various lesions did not materially change or delay the picture of the recovery outlined.

THE EXPERIMENTAL RESULTS. *1. The rôle of the splanchnic constrictor fibers in the rise of pressure during cerebral anemia:* Following the work of Claude Bernard in 1848 who showed that the section of the cervical cord caused a considerable fall of blood pressure, Bezold, Ludwig and Cyons (46), (47), (48), (49), (50) measured the magnitude of these changes and showed their dependence on the integrity of the splanchnic system. There was thus demonstrated the relation of the blood pressure changes to the level which is maintained after the continuity of the cord with the brain has been interrupted.

Mall (51) showed that frequently 27 per cent of the blood in dogs was transferred by the splanchnic system, thus explaining the great increase of volume in the extremities during rises of systemic pressure (52). Edwards (53) calculated that 85 cc. of blood in dogs were translocated under splanchnic stimulation. In spite of its probable involvement in the powerful vasomotor response of the anemic rise, very little direct evidence for its participation has been obtained. Hill's (46) reference to the splanchnic nerves in cerebral anemia is, so far as can be ascertained, largely by way of implication. For asphyxia itself both V. Anrep (54) and Cathcart and Clark (55) have argued for considerable splanchnic participation from the dependence on the central nervous system of the adrenalin release obtained. Finally, some indirect evidence for splanchnic nerve involvement has been obtained by section of the spinal cord in cerebral occlusion. Nawalichin (56) found that the vasomotor changes following obstruction of the cerebral circulation were practically obliterated when the cord had been sectioned in the cervical region. The same observation was made by Stewart (28).

In order to obtain any exact, or possibly even quantitative, evaluation of the actual involvement of the splanchnic system, other factors concerned in the maintenance and change of blood pressure must be isolated. Three factors in the nervous regulation must above all be properly controlled. These are (*a*), the indirect effect of the activity of the skeletal muscles; (*b*), the influence of the cardiac innervation; and (*c*), the non-splanchnic constrictor (or possibly dilator) fibers in the vasomotor system.

a. The influence of the skeletal muscles in the anemic rise. The older authors, Mayer and Couty, used curarized animals, rabbits and dogs, for their experiments on cerebral occlusions, and reported anemic

rises as great in magnitude and duration as those recorded by Stewart (28) or those herein obtained. The relative volume of blood held in these animals within the splanchnic system, as compared with that controlled by the somatic innervation is, however, somewhat different from that in cats. Little experimental attention was here given to this problem. In one animal, however, curare was injected and a vigorous anemic response was obtained. The occlusion time was normal (3 minutes); the anemic increment, however, was below the average, being only 80 mm. In another cat, both sciatics and the brachial plexuses on both sides were divided. Pressure did not fall after the lesions. Both stellate ganglia were then removed. The animal gave an anemic increment of 100 mm. Hg.

It seems accordingly that the muscular factor is of no primary significance in either the initiation or the maintenance of the anemic rise. The fact that no great depression of the level of blood pressure results in spite of extensive elimination of muscular innervation is interesting in comparison with subsequent results, and effectively contrasts the influence of skeletal innervation and visceral innervation on blood pressure.

b. The influence of the cardiac innervation on the anemic rise The influence of the cardiac nerves on the anemic rise may be exerted in either of two ways. The change in rate and amplitude of the heart beat may affect the output per minute as emphasized by Tigerstedt, (57) or afferent impulses aroused within the heart may affect reflexly the efferent cardio-vascular innervation as discussed by Hill (59). It is conceivable that in either of these ways, or both, the heart may influence significantly the level of blood pressure.

Frank (57), mathematically, and Erlanger (58) by sphygmomanometric measurements, have attempted to show that the output of the heart remained a constant, or in other words that pulse pressure times pulse rate remains a constant. Wickwire (60) has shown that the usual compensatory changes in heart rate to a change in the systemic blood pressure may be absent in deep anesthesia or in cases of restriction of the volume of blood flow to the brain. Under normal circumstances, Erlanger's statement probably holds true, but may not necessarily apply under critical conditions.

1. Effect of the vagus. Mosso (61), Couty and Stewart found that following the first short rise in blood pressure (which in the intact animal is never very great) there is a considerable slowing of the pulse. As long as this slowing of the pulse persists, pressure ceases to rise, and

is indeed often lowered. After about half a minute of this effect, the heart seems to break away from this retardation, and the beat is, if anything, accelerated and pressure immediately rises to the maximum level which is maintained until its final fall. The slowing of the heart rate and the depression of blood pressure gives the anemic rise its typical double crest. Both Couty (16) and Stewart (26) saw this double crest disappear on section of the vagi, leaving a smooth curve, which attains its maximum height somewhat more rapidly, but is not otherwise greatly altered in time or intensity.

Bilateral vagotomy has been done only incidentally to other lesions. The results confirm the earlier findings.

2. Excision of the stellate ganglia. Section of the accelerators as the only lesion was undertaken in five cats, all except one dissection being made in the open thorax under artificial respiration. In all cases the entire stellate ganglion was removed. The mass of nervous tissue was secured by a hemostat and this then cut away from all the connections by which it was held, until the hemostat could be removed without tearing. All the records therefore give a picture of the effects obtained by excision of the entire ganglion including, of course, those additional accelerator fibers recorded by Ranson, Spadolini and Wickwire (60), which reach the stellate ganglion by way of the superior cervical ganglion.

Hunt (62) recorded a loss of pressure on section of the stellate ganglia. Wickwire found a considerable loss (60 mm.) on their section, when this was undertaken without a previous vagotomy. In two cats, 1 and 3, a similar depression was noted. In cat 2, however, the fall was only 20 mm. In cat 7, in which pressure was already very low, no change at all was noted.

Section of the accelerators on both sides seems, like double vagotomy, to have a typical effect on the contour of the curve. It also tends to obliterate the double nature of the curve, which then more closely approaches a single peak. Characteristically, section of the accelerators imparts to the anemic rise a marked plateau effect. After a relatively restricted latent period, pressure rises sharply to its maximum level (fig. 1, occlusion 2), near which it is maintained until just prior to its final fall, when it may again strike the greatest height. The anemic increment of pressure for the five cats examined lay between 120 and 160 mm. Hg. Such a vagus effect as made itself felt, curiously enough, appeared somewhat later than when the accelerators were intact, and the slowing was recorded at the crest of the wave at a very

high level of blood pressure. Occasionally a sharp depressor effect may be recorded, which is rapidly compensated for; this effect gives an M-shaped appearance to the curve. On the whole, with the stellate ganglia excised, pressor responses are more promptly executed and longer maintained. In six additional cats, section of the accelerators was complicated by other lesions. In the two cases in which it was preceded by low section of the sympathetic chain, an anemic increment of 80 mm. was obtained in each.

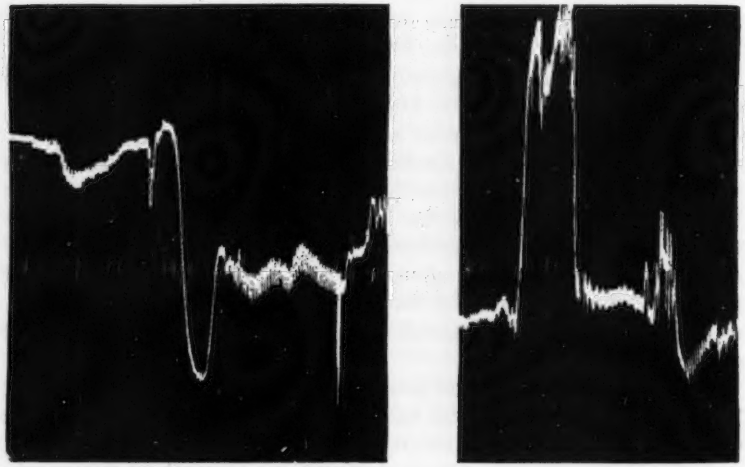

Fig. 1. Cat 4; Occlusion 1. Cerebral anemia, anomalous contour of curve. In this, great fall of pressure replaces the rise ordinarily obtained. The levels of blood pressure before anemia, after anemia and after recovery of bulbar function are shown. Head arteries were released immediately after the rise of pressure, in which the pre-occlusion level was partially recovered. Pressure fell subsequently as low as the lowest point obtained during anemia, but regained 90 mm. above this level with the return of bulbar function.

Occlusion 2: Cerebral anemia; record following excision of both stellate ganglia. When anemia is induced, pressure is 50 mm. lower than after recovery of bulbar function, prior to section of the stellates; M-shaped curve, showing sharp immediate rise of pressure, almost to its maximal height; vagus effect appears at crest of wave. Temporary recovery on release of head arteries, followed by fall to lowest level of pressure (55 mm. Hg. above base line). This low level was three times reached in this animal. Final rise of pressure on renewed return of respiration and other medullary activities.

Each occlusion occupied 3 minutes.

3. Excision of the entire cardiac innervation. In three cats the section of both vagi and accelerators was undertaken without any previous lesion. In two of them, section of the vagi was undertaken first, and in both cases a rise of 20 mm. obtained. Subsequent section of the stellates did not appreciably lower (by more than 5 mm.) the original level. The order in which the section of the cardiac nerves is carried out is, therefore, significant for the general level of pressure, and is again in agreement with Miss Wickwire's findings. Several successive curves were obtained from cat 5. The anemic increment was in these cases somewhat reduced, increments of 80 to 100 mm. being obtained after elimination of all the extrinsic cardiac nerves. When all cardiac nerves were sectioned, the curve tended to be smooth, the initial acute rise not being at all delayed. No change in the occlusion time was noted.

Recovery from occlusion after excision of one or both sets of the extrinsic cardiac nerves was uniformly obtained. The time interval of recovery was in no way different from that in normal animals.

In additional cats to be mentioned later, excision of the extrinsic innervation was preceded by a low section in the sympathetic chain. One animal gave an even higher anemic increment (125 mm. Hg.) than is usually obtained after section of the cardiac nerves alone.

In all the curves of reaction to anemia from animals with denervated hearts, pressure was not uniformly maintained at the maximal level. In two cases the pressure dropped immediately; in the rest (4 cases) a plateau was maintained.

4. Effect of the cardiac innervation on the anemic rise. Neither lesion of the cardiac innervation, as a whole, nor of the vagi, nor of the stellate ganglia separately, greatly affects the blood pressure response. Its duration seems to be fairly constant for the given individual tested. Excision of the entire cardiac innervation may reduce the anemic increment in some cases, but the reduction when it occurs does not seem to be considerable.

However, the cardiac nerves seem to have considerable influence on the level of blood pressure in the more detailed relations of the anemic rise, especially in the early part of the reaction. From the results of the section of the accelerators, particularly the abruptness with which an intense rise appears immediately on occlusion of the head arteries, it seems that the conception of the action of the accelerators must be extended. Marey asserted in 1881 that with the vagus intact no very great rise of pressure can be obtained. Indeed, as long as the vagi are

functional the maximal anemic increment is not immediately obtained, and cannot be reached in the early part of the occlusion unless the vagi be sectioned. The same seems to follow also for the accelerators since, when they are removed, the vagus cannot prevent the immediate and considerable augmentation of pressure. In the earlier part of the anemic response, the combined action of the entire cardiac innervation seems to effect a considerable check on the rapid rise of blood pressure. This may be due to afferent or efferent impulses, but the accelerators seem to be involved as well as the vagi.

The relations of the cardiac innervation to the second rise of pressure are not so clear. Stewart (28) attributed this in part to accelerator fibers in the stellate ganglion, and possibly in the vagus, but recently Stewart and Rogoff (63) have demonstrated the possibility of producing

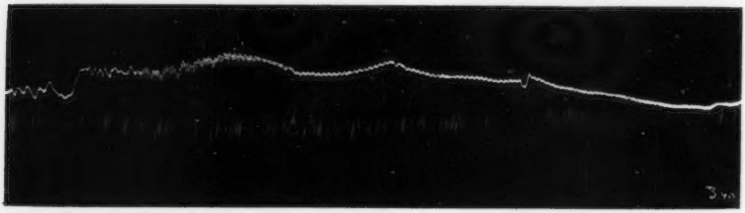

Fig. 2. Cat 23; cerebral anemia. Splanchnic nerves sectioned at their entry to coeliac ganglia. Occlusion time 3 minutes. Skeletal convulsions and respiratory spasms evident. The only factors in the vascular reaction recognizable in the tracing are the changes in heart rate. This is accompanied by a very slight change in level as the heart is breaking away at the usual time from its slow rate. Two respiratory gasps are later imposed on the tracing.

cardiac acceleration by sciatic stimulation even after the heart is completely denervated. In this series of experiments the rise appears very definitely in cats with accelerators removed and vagus intact. It must, therefore, be referable to vasomotor or endocrine effects under these conditions. Ordinarily, there is no break in the curve after double vagotomy, the fall due to vagus slowing being absent. In two cats, however, such a second rise has also been seen when the heart was completely denervated. It seems that the cessation of the vagus effect, while undoubtedly significant, is only one of the factors involved.

In the absence of the influence of the cardiac nerves on the initiation and maintenance of the reaction to cerebral anemia, it seems that we must look to the vasomotor mechanism itself.

It is interesting to note, however, that when the animal is no longer intact, and the peripheral resistance has been markedly lowered by a high transection of the cord, these relations are changed. Yates (36) has observed that cats which showed a considerable anemic rise after recovery from such a section, completely lost their ability to react to cerebral anemia following a subsequent excision of the stellate ganglia. However important for all practical purposes the vasomotor control may be, the considerable involvement of cardiac factors in the integrated response, particularly in the event of injury to the vasomotor nerves, must not be overlooked.

c. Influence of the splanchnic nerves on the anemic rise. The wide distribution of the splanchnics, gives a possibility for various lesions within the system. Section of the splanchnics was therefore undertaken *1*, in the base of the sympathetic chain before leaving the thorax; *2*, in the abdomen, just prior to their entrance into the coeliac ganglion; *3*, in various levels of the spinal cord in the thoracic region.

The anatomical relations of the splanchnic outflow in the cat have been described by Langley (64), who concludes that the fibers destined to enter the splanchnic nerves leave the cord in large part below the level of the sixth thoracic, though occasionally fibers can be traced at the level of the fifth and even fourth thoracic. Langley's statement appears based only in part on his own observations, and is largely founded on the work of other investigators embodied in the papers quoted. Several authors included higher levels for the effects studied based on experimental rather than anatomical evidence though all have stated that the effect elicitable is relatively slight. Bayliss and Starling gave 3rd thoracic as supplying the portal circulation, Bradford, the 3rd thoracic as supplying the kidney; and Schäfer and Moore, 3rd thoracic as supplying the spleen.

In a more recent study on cats Ranson (65) has re-investigated the problem. He confirms Langley's findings and considers the 4th thoracic the highest limit of the splanchnic outflow. Ranson's material, however, was in part restricted to animals in which only the levels below the 6th thoracic were examined. Ranson has investigated further the level at which the splanchnic nerve leaves the sympathetic chain. In far the greater number of cases (13 out of 17) the nerve was given off between the 1st lumbar and 13th thoracic ganglion, in the remaining four cases, the nerve left between the 1st and 2nd lumbar ganglia. The relation of this branching to the diaphragm was not stated.

1. Lesions within the splanchnic outflow: Section of the sympathetic chain; thoracic section of the splanchnics. In 12 animals the splanchnic outflow was interrupted in the lower thorax. Under artificial respiration, a low midventral incision was extended bilaterally on either side of the diaphragm, and the lungs held back while the sympathetic chain was isolated and sectioned.

Section of the sympathetic chain below the level of the 8th or 9th thoracic vertebrae usually gives a very marked fall of pressure. When the splanchnic branch from the sympathetic chain itself is cut, this depression amounts at least to 80 mm. Hg. In spite of this low level of pressure, spontaneous respiration is not usually lost, and when ether is reduced, eye reflexes and other skeletal responses are readily elicitable. The condition of the animal, however, is precarious, and prolonged operative manipulations with too great a depth of anesthesia will readily cause complete loss of the bulbar responses. This precarious condition is in fact met with in all extended lesions within the splanchnic system, and offers some difficulty in the further manipulation of the animals.

Occlusion of the head arteries in this series generally gave a relatively vigorous response. The intensity of the response varied, the degree of variation from the normal being dependent apparently on the nature of the lesion.

Group I. In these animals section of the sympathetic chain was undertaken in its lower levels, post-mortem examination showing no lesion above the level of the 8th thoracic. In two of these animals autopsy showed the lesion incomplete on one side, thus amounting largely to a unilateral injury. The anemic response obtained in four of these animals was very considerable, the values being 100, 120, 140, 150 mm. Hg., respectively. The contour of the curves was typical of the normal anemic responses, and the rise of pressure easily over-reached the original control level of blood pressure. All these cats showed a normal recovery from the occlusion. In nos. 12 and 15, excision of the stellate ganglia was done subsequent to recovery and a third occlusion obtained. Cat 15 that had shown an unusually vigorous response in its first occlusion gave an increment of 125 mm. Hg. after excision of both vagi and both stellates. The thoracic chain was sectioned at the level of the 8th and 9th thoracic on one side, between the 10th and 11th on the other. Cat 11 was slightly different. The original depression of blood pressure after section of the chain was 80 mm. Hg.; the anemic increment was somewhat reduced, amounting only to 70 mm. so that

the anemic rise fell short of reaching the original level. The cat recovered, however, and subsequently made up the 10 mm. difference in an anemic rise obtained after the stellate ganglia had been excised. The greater splanchnics may have been involved in this case.

Group II. This comprised the remaining 7 cats of the series. In all these animals a complete bilateral section of the splanchnic nerves was done in the thorax between their branching from the sympathetic chain and before their entry into the diaphragm. On cutting the splanchnic nerves the initial fall of pressure was great, averaging 80 mm. In four cats the effect of occlusion was well marked, the curves differing from the normal only in a slight reduction of the anemic increment of blood pressure, this being 70 mm. in three cases. In these cases also pressure did not reach the level held prior to section.

In the three remaining cats of the series a still greater depression of the anemic response was obtained. Cat 20 gave a most complete picture. The anemic rise reduplicated all the characteristics of the normal response on a smaller scale. A vagus effect appeared prominently. The maximum anemic increment of pressure in these experiments was 40 mm. When pressure fell spontaneously after occlusion it reached the identical level maintained after section of the splanchnics prior to occlusion. Low section of the spinal cord at this time induced a further fall of only 10 mm. Hg. In cat 22 the acclerators were also removed, and an even greater depression of the anemic response was obtained, the entire change of level on occlusion amounting to only 5 mm.

Cat 21 was slightly anomalous but yet highly instructive. The animal showed a great resistance to anemia, and it took some 15 minutes before the respiratory and vasomotor responses fully faded out. At first the record clearly approximated that of cat 20, an initial rise of 30 mm. being shown. With the persistence of the bulbar functions, however, there was reproduced on a different scale, the wide oscillations procurable in all animals difficult to asphyxiate. At first the vasomotor oscillations were slight and rather irregular, but they gradually developed into large and rapid waves in which the greatest excursion of blood pressure was developed, amounting to a fluctuation of 60 mm. at the height of the response. The level of blood pressure from which these oscillations developed was not raised, the whole response being simply recorded within this maximum variation of 60 mm. This offers a striking contrast to the analogous records of incomplete occlusion periods of similar length obtained in intact ani-

mals. In such animals the level of pressure shows similar oscillations, but these vary within a much greater range, usually approaching 200 mm. difference in level. No recovery of bulbar functions was elicited from any of these animals. That this was not the necessary consequence of a lesion at this level, but merely an indication of the precarious conditions of animals exposed to this double lesion, is shown in the following experiments.

Section of the sympathetic chain; abdominal section of the splanchnics. Although the blood pressure response is seriously reduced by section of the splanchnic nerves above the diaphragm, a slight degree of response still seems elicitable. It seems possible, however, completely to eliminate all rise of blood pressure as the result of bulbar anemia, while maintaining all other evidence of medullary activity, by section of the greater splanchnic nerves in the abdomen.

Dissection for the splanchnics in the abdomen was made by the method indicated in Sherrington's *Mammalian Physiology* (66). The incisions were made from the back, through the latissimus dorsi muscles, and the nerves were cut just before their entry into the coeliac ganglion. The identity of the nerves was first tested by electrical stimulation with shielded electrodes.

A striking example of the result of this section was obtained in cat 23. In this animal (fig. 2) the greatest excursion of blood pressure amounted to 10 mm., yet all other effects of occlusion were noted. An asphyxial effect on the vagi appeared in the pressure curve followed by a very slight improvement in the level. From this point on, however, pressure fell very gradually, until, at the end of 3 minutes, it remained constant. In this very gradual fall, pressure reached a level some 20 mm. below that of the original pressure before occlusion. After digital compression of the abdominal aorta, spontaneous respiration returned in this animal. When respiration had become completely reëstablished and a corneal reflex again obtained, the trachea was clamped. No asphyxial rise of pressure to speak of was obtained, the entire subsequent variation of pressure being well within 20 mm. Hg. Respiratory waves and some vagus effect were recorded; failure of the heart soon followed.

Section of the thoracic spinal cord. Section of the spinal cord was undertaken in 16 cats. The laminectomy was carried out immediately following tracheotomy, the wound temporarily closed by hemostats and the head arteries then prepared for ligation. Finally the cord was sectioned, and blood pressure allowed to reach a constant level

before inflicting any further lesions. Several successive sections of the cord were frequently carried out in the same animal before occlusion was produced.

Section of the thoracic cord was carried out at various levels. The effect of section varied considerably with the level of the lesion, and to some extent also with the individual animal. Certain results, however, are patent. Lesion in the lowest levels of the thorax elicited only a slight permanent fall of pressure, and did not seriously affect the anemic response. Lesions in the midthoracic, at the level of the 8th thoracic and 9th thoracic vertebrae were more apt to elicit a profound fall of pressure, and seriously to reduce the anemic increment. Lesions in the upper thorax also elicited a great fall on section and often completely

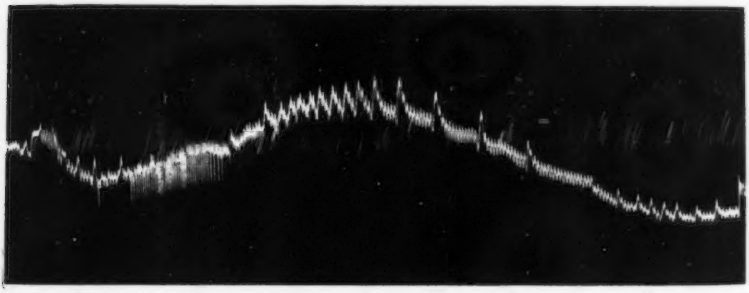

Fig. 3. Cat 30: cerebral anemia. Spinal cord sectioned at the level of the 5th thoractic verterbra. This reaction shows the features of the typical blood vascular response to anemia in every respect, but the level to which the maximal rise of pressure (second rise) attains. The anemic increment here is only 50 mm. Hg. Cardiac effects of slowing and acceleration recorded as usual.

abolished the rise of pressure. There were, however, certain individuals in which even a high thoracic lesion did not evoke a maximum fall of pressure, and in which a relatively vigorous response was obtained even after a high dorsal section. Accordingly the experimental material can be roughly classified into three groups:

Group I. Lesions in the lower thoracic region. Very vigorous responses to cerebral anemia can still be obtained from animals with a lesion at the level of the 10th to 12th thoracic vertebrae. Cat 25 with section at T 10–11 showed an anemic increment of 125 mm. Hg. In cat 24 an anemic response lasting over 5 minutes was obtained, in which the variation of pressure extended over 75 mm. Hg. The contour of

TABLE 1

Section of the sympathetic chain and thoracic section of the splanchnic nerves

CAT NUMBER	DATE OF EXPERIMENT	NATURE OF LESION	DECREMENT ON SECTION	ANEMIC INCREMENT	DESCRIPTION OF CURVE
			mm.	*mm.*	
11	Nov. 20, 1918	Chain sectioned left 9–10 T, right 8–9 T	60	70	Smooth curve, gradual ascent and descent
12	Nov. 19, 1918	Chain in lower thorax right lesion incomplete	No record	100	Normal double peaked curve
13	Nov. 29, 1918 Cat I	Chain in lower thorax left lesion incomplete	70	140	Smooth curve, gradual ascent and descent
14	Nov. 26, 1918	Chain cut above diaphragm	No record	60	Smooth curve, gradual ascent and descent
15	Jan. 28, 1919	Chain sectioned right 8–9 T, left 10–11 T	40	150	Typical curve, great rise long maintained, occlusion time—10 minutes
16	Jan. 14, 1919	Low section of chain and splanchnics	60	120	Typical double curve, pronounced vagus effect, rise long maintained, occlusion time—10 minutes
17	Feb. 1, 1919	Section of splanchnic nerves above diaphragm	80	70	Double curve, marked vagus effect, pressure does not quite reach original level

18	Jan. 7, 1919	Section of splanchnic nerves above diaphragm	No record	70	Double curve, marked vagus effect
19	Jan. 16, 1919	Section of splanchnic nerves above diaphragm	80	70	Double curve, marked vagus effect
20	Feb. 8, 1919	Section of chain and splanchnic nerves just above diaphragm	80	40	Marked vagus effect, further section of cord (low) shows loss of only 10 more mm.
21	Jan. 21, 1919	Section of chain and splanchnic nerves just above diaphragm	No record	60	Cat difficult to occlude, respiration fades out after 15 min. Great oscillations of pressure towards end of time
22	Nov. 29, 1919	Section of chain and splanchnics just above diaphragm	80	8	Corneal present just before occlusion, but no appreciable effect of vagus slowing, etc.

TABLE 2

Section of the spinal cord at various levels

CAT NUMBER	DATE OF EXPERIMENT	LEVEL OF SECTION	DECREMENT ON SECTION	TOTAL DECREMENT	ANEMIC INCREMENT	DESCRIPTION OF CURVE
			mm.	*mm.*	*mm.*	
34	Apr. 15, 1919	T 10–11	60	30*		No occlusion
		T 8– 9	30	60	60	Sharp ascent and descent, pressure just reaches height prior to last section
38	Mar. 18, 1919	T 10	78	44		No occlusion
		T 9– 7	20	60	50	Sharp rise marked vagus effect, with loss of 10 mm. pressure
		T 6– 2	10	70	5	Some slowing of the heart shown, no increment
31	Apr. 9, 1919	T 2	110	110	40	Double curve, sharp 2nd rise
35	Apr. 16, 1919 Cat II	T 6	55	55 40		No occlusion
		T 4	40	95	20	Depression of pressure only during occlusion; very slight effect
39	Apr. 16, 1919	T 8	68	68		No occlusion
		T 6	30	98	10	No appreciable effect although slight vagus slowing

* 30 mm. again recovered.

42	Oct. 22, 1920	T 8	100	100	20	Rather marked slowing
		Vagotomy	30 rise	70	50	Poor record, difficulties with manometer
44	Nov. 19, 1920	T 6	75	75	20	No marked vagus effect, respiratory oscillations show in curve
33	Apr. 21, 1920	T 8	30†	30	30	Marked double rise pronounced vagus effect
		T 6	20	50	10	Effect extremely reduced
24	Feb. 19, 1919	T 10-11	40	40	80	Great fall instead of rise (Cf. text)
		T 8- 9	80	120	30	Marked vagus slowing, curve very flat
		Splanchnic nerves cut	No further fall	120	30	Rise obtained on clamped abdominal aorta
25	Feb. 20, 1919	T 11-12	80	80	150	Absolutely typical curve, very sharp rise
29	Mar. 11, 1919	T 11	50	50	70	Marked vagus effect, pressure drops 40 mm. lower after occlusion
30	Mar. 15, 1919	T 11-10	10	Level completely recovered rise above control level		No occlusion
		T 9- 5	60	60	50	Very marked curve obtained, vagus effect, second rise accentuated

† Pressure only 80 mm. Hg. to begin with.

TABLE 2—*Concluded*

CAT NUMBER	DATE OF EXPERIMENT	LEVEL OF SECTION	DECREMENT ON SECTION	TOTAL DECREMENT	ANEMIC INCREMENT	DESCRIPTION OF CURVE
			mm.	*mm.*	*mm.*	
26	Feb. 22, 1919	T 12	No record		40	Vagus effect appears, curve highly reduced
28	Mar. 8, 1919	L 3	40			Marked vagus effect, pressure
		T 10	No further fall	40	30	Rises only 30 mm. but falls 30 more
		T 8	30	70		No asphyxial increment
40	Apr. 22, 1919	T 9–6	40		40	Very marked vagus effect
		T 6–5	20	60	50	Sharp ascent and descent, marked vagus effect
		T 4	No further fall		42	Sharp ascent and descent, marked vagus effect, pressure drops 30 mm. lower
		T 2	24	84	28	Clear double curve, vagus depression
		Sympathetic chain cut low in thorax	20	104		No asphyxial increment

this curve will be discussed below in connection with similar anomalous curves obtained from control records in other animals. In these cats a fall of pressure replaces the usual rise; the variation of level being of the same order of magnitude. In cat 24, despite the great drop of pressure, the original level was regained toward the end of the anemic response at the time usually occupied by the second rise of pressure.

Measurements of loss of pressure after section in the lowest levels of the thorax show a maximal total loss of 50 mm. Hg. Frequently only a few millimeters are lost. In cat 30 the 10 mm. lost after section at the 10th thoracic were completely recovered within 10 minutes, pressure rising even above the level recorded prior to transection.

Group II. Abolition of the anemic response. Lesions of the cord in the region of the 8th thoracic usually entail a rather severe effect; the loss of pressure following this section may amount to 80 mm. Hg. If the fall is as great as this, the anemic response is apt to be seriously diminished. Cat 25, which gave a very vigorous response after section at T 10, showed a further loss of 80 mm. when the cord was sectioned at T 8, the level falling 100 mm. below that held when the animal was intact. The anemic increment after section at T 8 was only 30 mm. Hg. The reduction of the anemic increment to a variation of pressure of only 30 to 40 mm. was seen in five other experiments, (cats 24, 30, 38, 40, and 42) in which section in the region of the 8th to 10th thoracic gave a considerable depression of the level of blood pressure and in which anemia of the bulb failed to evoke an increment of pressure greater than 40 mm. That the vagus is partially responsible for this effect is indicated by cat 42, in which an initial response after section in the 8th thoracic gave an increment of only 20 mm. This increment, however, rose slightly above 40 mm. in a subsequent occlusion after the vagi had been sectioned.

Section of the cord above the 8th thoracic in three animals, cats 35, 39 and 44, gave a very marked fall of pressure in all these cases and no anemic response greater than 30 mm. was obtainable in any one of them. In cat 44, only one section was made at T 6, and no anemic increment at all was obtainable after occlusion. In the other two animals several successive sections were undertaken before occlusion was tested. In cat 39, the first section was carried out at T 7; this was followed by a fall of 55 mm.; 40 mm. more were lost in successive sections ascending to the level of T 4. In cat 35, 80 mm. were lost by section at T 8, and only 20 mm. more by successive sections to T 6. The level of pressure above base line from which only a minimal sub-

sequent fall occurs under further manipulations, lies between 35 to 50 mm., the residual pressure maintained by the spinal cord alone.

Group III. Retention of an anemic effect. The midthoracic region is apparently not critical for vasomotor responses in all animals. In cat 34 section at the 8th thoracic gave a loss of only 30 mm. Hg. and an anemic increment of 68 mm. Hg. was obtained. The relatively slight loss of pressure following section at T 7 in cat 39 above mentioned also shows that in some animals the higher levels of the cord are of great importance.

However, the most significant indication of participation of the upper levels of the cord in conveying fibers significant for the vasomotor response was obtained in four additional animals. A strikingly complete anemic rise was obtained from cat 30, in which the cord had been sectioned as high as T 5 (fig. 3). The anemic increment here was 54 mm. In cat 38 a well-maintained response of 44 mm. was obtained on occlusion after section at T 6. In cat 31 a rise of the same magnitude (40 mm.) was obtained after section at T 2. The level maintained after section at T 2 prior to occlusion was 65 mm. Hg. above base line and did not reach the level of 50 mm. until after occlusion. An interesting record of the potency of the higher levels in certain individuals for both the maintenance of blood pressure and its variation is best given in the following protocol—cat 40.

Condensed protocol, cat 40 (pressure here given in level above base line) April 22, 1918

Tracheotomy, blood pressure, cannula, laminectomy, head arteries prepared for ligation.

2:30 Control blood pressure—130 mm. Hg.
2:35 Section of cord at T 8
2:40 Level of blood pressure—118 mm. Hg.
2:45 Section of cord at T 6
2:47 Level of blood pressure—90 mm. Hg., total depression of pressure—40 mm.
2:48 Corneal reflex
Occlusion. Sharp rise of pressure to 114 mm. drop to 90 mm. Hg., great vagus effect, rise again to 130 mm.: *anemic increment—40 mm.*
2:51 Pressure released before spontaneous fall began, gasps immediately return, fall to 80 mm.
2:57 Respiration reëstablished, section of cord at T 5
2:58 Level of blood pressure—70 mm. Hg., total depression of pressure—60 mm.
2:59 Corneal reflex, *occlusion:*—incomplete
3:01 Further manipulation of clamps, immediate rise of pressure to 114 mm., *anemic increment*—44 mm.
3:04 Released before spontaneous fall, pressure drops to 50 mm. Hg., but immediately begins again to recover

3:05 Gasps return, pressure continues rising
3:10 Pressure reaches 90 mm. Hg. again, regular waves in blood pressure curve, respiration reëstablished
3:17 Section of cord at T 4, pressure drops to 70 mm.
Corneal reflex, *Occlusion:* sharp rise of pressure to 100 mm. fall to 60 mm. during vagus slowing, subsequent rise to 112 mm.; *anemic increment*—42 mm.
3:23 Pressure released before spontaneous fail, drops sharply to 44 mm. but immediately begins to rise again
3:25 Gasps return
3:33 Pressure at 84 mm. again, respiration reëstablished
3:39 Section of cord at T 2, pressure falls to 46 mm. Hg. total depression of pressure, 84 mm.
3:40 Corneal reflex, *Occlusion:* initial rise to 64 mm. Hg. fall to 30 mm. during vagus slowing, second rise to 74 mm. Hg. *anemic increment*—28 mm.
3:42 30 Head arteries released before spontaneous fail, pressure immediately falls to 20 mm. but again regains level
3:45 Pressure has reached 54 mm., gasps return, further rise to 60 mm. respiration reëstablished
Thorax opened, artificial respiration administered
4:05 Sympathetic chain cut in midthoracic, pressure fall to 40 mm. total depression of pressure—90 mm.
4:07 Corneal reflex: occlusion, rise to 52 mm. slight fall, rise to 48 mm. *anemic increment*—12 mm.
4:11 Release of head arteries, pressure drops to 30 mm.
4:14 Gasps return, pressure rises to 40 mm. respiration reëstablished
4:55 Splanchnics cut in psoas muscles, no effect on blood pressure, no recovery of level or return of respiration
5:10 Artificial respiration intermitted, pressure drops to base line.

Effect of the splanchnic constrictor fibers on the anemic rise. The burden of the anemic response seems to lie in the vasomotor apparatus, and, if the evidence of these experiments is adequate, almost exclusively on the splanchnic constrictor fibers. Peripheral section of the splanchnic nerves, with its great depression of blood pressure, and the subsequent inability to obtain any anemic increment whatever, speaks strongly, almost unequivocally, for such an interpretation. Additional evidence for the importance of the splanchnic pathway for the vasomotor changes during anemia is the relation of the level of blood pressure after section within the splanchnic outflow to the anemic increment elicitable on occlusion. The data show quite clearly that *the greater the initial depression, the less powerful the response.*

This very definite grading of the blood pressure level to the magnitude of the anemic rise gives a further insight into the anatomical relations of the splanchnic outflow. In the cats examined the greatest

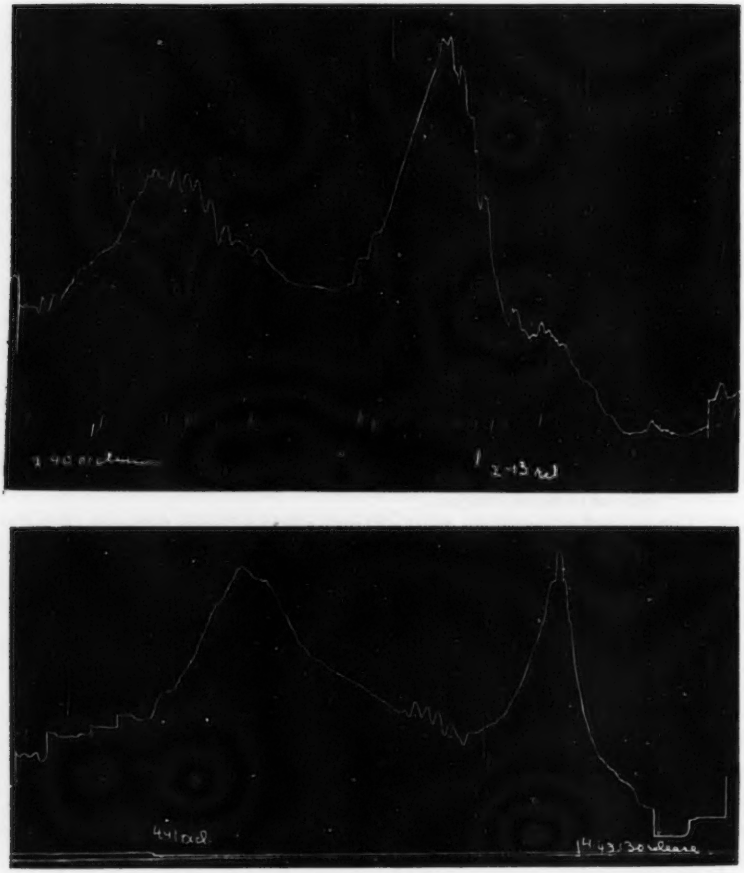

Fig. 4. Cat. 45. Repeated cerebral anemia; double vagotomy; 10th successive occlusion; dissociation of reaction curve into two distinctly separated peaks is shown fully established. Second peak in this occlusion is still considerably greater than the first. Pressure falls very low at the end of the response (30 mm. Hg. above base line). Each curve occupies approximately half the period of the reaction.

Seventeenth successive occlusion: Dissociated curves show little difference in time relations compared with those obtained 2 hours earlier. Difference in contour found in the greater emphasis of the first as compared with the second peak of pressure. The rise in level prior to occlusion represents the recovery of blood pressure after the preceding occlusion following the return of respiratory gasps.

average ouflow from the cord to the sympathetic chain is apparently in the region of the 6th to 8th thoracic. Yet the outflow is not restricted to the lower thoracic region, for fibers in the higher thoracic region even as high as the 1st or 2nd, have in these experiments been found of some importance both for the maintenance of the level and the changes of blood pressure. Such a curve as that obtained from cat 30 (fig. 3) shows convincingly that in some animals a good proportion of fibers leave the spinal cord to enter the sympathetic chain above the level of the 5th thoracic vertebra.

While therefore the anatomical findings of Langley and Ranson for the average level of outflow have in the main been verified, the involvement of higher levels already indicated by the various physiological researches quoted by Langley has received a rather striking confirmation.

In such an instance, the physiological evidence may well have precedence over the anatomical, for whereas a small bundle of fibers is most difficult to stain and trace microscopically, a weak physiological effect, when definitely isolated, is quite unmistakable. The involvement of these fibers from the higher levels within the splanchnic system, becomes of particular importance when the entire burden of the splanchnic function is restricted to these levels. Since the very high outflow presumably goes by way of the stellate ganglion, it is necessary to differentiate between the effect of the splanchnic fibers proper and the accelerators. However, in these cats in which an abdominal or high spinal section completely abolished the rise, the cardiac nerves seemed completely impotent against the lowered peripheral resistance.

Accordingly, the vasomotor impulses that travel through the splanchnic nerves to the coeliac ganglion may leave the cord as high as the first or second thoracic. They may, however, stay in the cord throughout the thoracic region and leave it even below the diaphragm. Ranson's results on the very low level at which the splanchnic nerve leaves the chain in cats, is in close agreement with these findings.

There thus appears to be a double pathway for the splanchnic outflow in the thorax, one within the cord, the other outside of it. The outflow of the splanchnic fibers from the cord to the sympathetic chain seems distributed over the entire thoracic region, the relative distribution varying from one individual to another.

In this light the impossibility of abolishing the anemic rise by a section which implicates only part of the splanchnic system is explicable. The wide distribution of the splanchnic fibers would make it difficult

to compromise the response by a definitive lesion. Such a lesion could, it seems, be secured only when the section falls sufficiently far out in the periphery or sufficiently high up in the cord, *definitely to interrupt the conduction pathways from medulla to coeliac ganglion.* Unless this interruption is accomplished, the fibers that are left in continuity with the medulla and the periphery are able to initiate an anemic rise which, even if considerably diminished in intensity, repeats all other characteristics of the usual vasomotor response.

To the splanchnic innervation, therefore, the most significant factors in the blood vascular reaction to cerebral anemia can be attributed: the initiation of the rise and the level which this reaches. Since these factors can be controlled by differential lesions within the splanchnic system, the influence of the non-splanchinc vasomotors may be neglected for the purposes of the present survey.

On the influence of the splanchnic system on the maintenance of the normal level of blood pressure: The splanchnic fibers seem involved when the level of pressure is above 50 to 60 mm. for unless a complete interruption of the conduction path from medulla to coeliac ganglion has been demonstrated, pressure returns to a higher level the height depending apparently on the number of fibers in the splanchnic system remaining functional. The level of 50 to 60 mm. is that shown by Mayer, Couty and later workers to be that maintained by the spinal cord alone. Yates also finds this level to be approximately that reached by blood pressure after recovery (2 to 32 days) from high transection of the spinal cord at 8th cervical to 5th thoracic. Her average level of pressure lay somewhat lower than this, between 40 to 50 mm. From Pike's and Langley's studies this residual spinal level appears rather as a skeletal or somatic, than as a vascular or sympathetic phenomenon.

The difference between the residual spinal level and the normal one—a difference of 80 to 100 mm.—would therefore appear as accounted for largely by the action of the sympathetic neurones within the splanchnic system. When the range of variation during anemia is examined, this is seen to be three to four times as great in the animal with splanchnics intact as in the animal which is largely dependent on its skeletal musculature. The variation of pressure in cats with low thoracic section of the sympathetic chain is greatly restricted where the animal was highly resistant to anemia and a period as long as 15 minutes elapsed before the processes activated by the higher levels ceased. Furthermore, as long as the splanchnic system is functional, pressure does not drop below the level of 50 to 60 mm. Hg., however great the variation

of pressure and no matter how exigent the inimical conditions. This is well illustrated by the variation of pressure noted in figure 3. The protocol of cat 40 where pressure in maintained in excess of 60 mm. until after a section of the spinal cord at the level of the 4th thoracic, also emphasizes the relation of this level to splanchnic activity.

On some anomalous curves. In a relatively large number of cats (8 in 60) a depression of blood pressure was obtained on occlusion instead of the usual anemic increment. This depression of the level of blood pressure was great, approaching the order of magnitude of the usual positive effect. In six of these cats, pressure fell 100 mm. and more below the original level of blood pressure. Most of these curves represented control occlusions, one example of which has been figured (fig. 1, occlusion 1) in which no previous lesion had been inflicted the vagi being intact in all cases. In one case, cat 24, also mentioned, this depression appeared after low section of the spinal cord. In these cats on occlusion there followed no initial increment, or only a very slight increase in the level (5 mm.). Pressure then continued constant for some 20 seconds. Following this a great and very rapid fall of blood pressure set in from which recovery occurred at about the time ordinarily occupied by the second rise of blood pressure. In this recovery from the low level of blood pressure, however, pressure approached but never completely attained the original level observed before occlusion.

The magnitude of the effect might argue for the involvement of the splanchnic system. As such, it might be aroused by an afferent excitation of the depressor fibers in the vagus. The relation of the depressor to the splanchnic system and also to the discharge of adrenalin (which would of course be involved in all splanchnic excitation) has been discussed by Ludwig and Cyon (48) and Oliver and Schäfer. Bayliss (67) has dealt with the antagonism of asphyxia and depressor stimulation.

On the other hand, the depression of blood pressure, instead of being due to the cardiac innervation set into action through an afferent channel, might be affected directly through a change in the minute volume of the heart, especially under changed conditions within the vagus system. Wickwire (60) has particularly noticed that different degrees of the depth of anesthesia gravely influence the changes in the level of blood pressure due to the vagus system.

II. Relation of the adrenal glands to the rise of pressure during cerebral anemia. The extensive involvement of the splanch-

nic system in the anemic response makes the activity, or some product of the activity, of the adrenal glands of considerable significance for the problem of its control. Following the discovery by Oliver and Schäfer (68) of the pressor action of injected extract of adrenal tissue, workers have tended to emphasize the close physiological relation of the glands and their pressor activity to the splanchnic system. The literature is extensive (69), (70), (71), (72), (73), (74), (75), but it will not be reviewed at this time. Nor will the literature on the liberation of adrenalin (75 to 90) be considered here. The evidence for the participation of adrenalin in the response to asphyxia and other conditions of stress is also extensive (91 to 98), but its consideration will be left for a later paper. The relation of the contour of the typical curve obtained on electrical stimulation of the splanchnic nerves to the adrenals, and also to the cardiac mechanism, has been dealt with by several authors (104), (105), (106), (107). A further analysis of this contour is also postponed.

Effect of repeated occlusion on intact cats. Elliott's assumption (75) that adrenalin is consumed under conditions of stress makes it conceivable that the rapid repetition of so radical a procedure as arterial occlusion could influence the amount of circulating adrenalin. Since the relation of adrenalin to the myo-neural junction has been experimentally demonstrated, Professor Pike has suggested that, physiologically, it may be associated with the process of conduction from sympathetic nerve fiber to smooth muscle, and directly or indirectly with the processes of excitation in smooth muscle. The work of Keith Lucas would suggest such a possibility (108). Accordingly, such an increase of activity of sympathetic nerve and smooth muscle as accompanies cerebral anemia should lead to a more rapid consumption of adrenalin. This conclusion follows from Elliott's hypothesis of the consumption of adrenalin. The procedure of repeated occlusion has accordingly been attempted first in intact animals in order to reach a control condition of maximal exhaustion of circulating adrenalin, and then in animals in which the adrenal glands had been permanently ligated.

The great resistance of the animals to repeated occlusion has been frequently demonstrated in the experimental material already given. Numerous other evidences of the relative indefatigability of vasomotor responses are found in the literature. Notable here are the analogous experimental conditions in the work of Cushing (99), who found that the process of raising the blood pressure by increasing the intracranial tension, and thus also inducing a partial anemia, could be repeated

indefinitely. The difficulty of inducing fatigue of the central vasomotor cells under normal conditions has been discussed in various connections by W. T. Porter (100), (101).

The experiments already described in this series on repetition of occlusion have been complicated by the infliction of lesions in the splanchnic system so that the actual ability of the animals to withstand repeated occlusions, and the effect of this procedure on the anemic response, was not clear. Furthermore, not more than six or eight successive occlusions at most were obtained. Accordingly, in five cats the effects of repeated occlusion were tested. Occlusion was done and when the final spontaneous fall of pressure occurred, the clamps were promptly released and recovery awaited. The corneal reflex was used as before as an index of returned bulbar activity. With its elicitation clamps were again adjusted on the head arteries, and this process repeated several times.

If the occlusion period was not too long maintained in any one closure, it was possible to repeat the procedure practically indefinitely. In the three most striking experiments, cats 45, 46 and 48, the experiments had to be halted arbitrarily because of extraneous reasons, the time consumed being too long. Cat 45 yielded 18 successive occlusions (fig. 4); cat 46, 13 successive occlusions; cat 49, 11 successive occlusions. These experiments lasted over 3 hours in addition to the time necessary for the preliminary operative manipulations which always consumed over half an hour. Cat 46 was intact, cat 45 had suffered double vagotomy, and in cat 48, (fig. 5A) both stellate ganglia had been removed. No marked difference in the behavior of these cats under the test could be noted. In fact, the cats showed a remarkable constancy in behavior. The characteristic occlusion time—2 to 4 minutes—in each individual was retained with considerable uniformity throughout each series. Furthermore, the time needed for recovery of the bulbar functions after release of the arteries was almost uniform for each cat examined. The recovery time which, on the whole, may be said to vary directly with the occlusion time, did not in all cases follow this relation. Cat 46, which gave a constant occlusion time of 2 minutes usually showed a recovery of a corneal reflex within 7 minutes subsequently. Cat 45, however, (vagotomized) invariably showed a 20-minute interval between occlusions. In this interval a well-marked recovery of blood pressure was noticeable and, with the return of respiration, blood pressure rose at least from 60 to 80 mm. above the level after occlusion, before a corneal reflex was

obtained. The average level between occlusions was relatively high, pressure seldom falling below 60 mm. Hg.

The first four or five occlusions obtained differed in no very striking detail from control occlusions. The main change from the type of these earlier occlusions appeared gradually. This was a slight delay in the appearance of the first rise in pressure and a gradual increase in the magnitude of this first effect. The fall of pressure from this first level also became more pronounced; pressure dropped to increasingly lower levels at this time on successive occlusions. By the time of the seventh or ninth occlusion the emphasis on this first part of the curve became so well marked that the entire response appeared more as two separate curves rather than one, the two summits in the tracing being very symmetrically distributed both in time and space. The fall of pressure following the initial rise was so great in some of the animals as to approach the base line very closely, dropping to a level of only 10 to 20 mm. Hg.

The characteristic new contour of the rise, once established, is retained in all subsequent tracings in the same animal with great uniformity (fig. 5B). The latter part of the series of occlusion records accordingly shows this new type of anemic rise. The marked drop in the double curve is quite different from the dip due to vagus action seen in the ordinary control pressure curve of anemia. It comes much later (fig. 4); it is also decidedly more abrupt and greater. In fact, it seems much more like an actual collapse of blood pressure. It appears uncomplicated by slowing of the heart. The very definite time relations established in these dissociated curves are striking. Indeed, the supplementary rise, once it has become separated from the initial rise by the marked temporary collapse of blood pressure, is recorded at exactly the same point in all later occlusions obtained in a given animal. This time closely approximates half of the occlusion time of the animal in which it appears, namely, at 1 minute in cats of 2-minute occlusions, and so forth. A decrease of the anemic increment was obtained in the course of the repetitions. Rises of 80 to 100 mm. gradually replaced the original increment of 120 to 140 mm.

One further observation on these cats is worth noting. Post-mortem examination showed that the blood of these animals failed to clot readily. It frequently flowed freely from the carotid artery when the cannula was removed. In a prolonged dissection in one animal, the blood flowed freely from every rupture of a large vessel, even as late as one hour after death.

Effect of repeated occlusions in cats deprived of adrenal glands. The procedure of repeated occlusion in the same animal was undertaken in a final series (six cats) in which both adrenal glands were ligated. In each of these animals one control occlusion was made, then by means of dissection through the latissimus dorsi (double incision from the back) the adrenals were isolated and secured by ligatures. No significant fall of pressure was obtained immediately on ligation of the adrenals, thus confirming the observation of Hoskins and McClure (102) and Young and Lehman (103). Following this, the procedure was

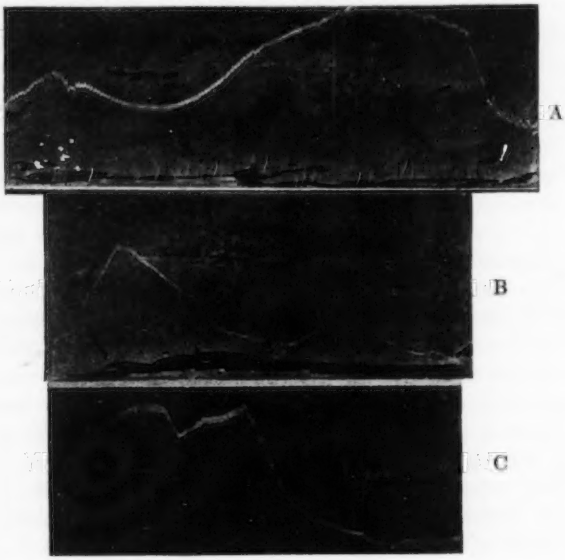

Fig. 5 A: Control curve of 3 minute occlusion. Ligation of the head arteries in the intact and fresh animal. Initial rise is followed by a depression level coming together with a slowing of the heart. High level of pressure maintained throughout the response.

B. Cat 48: Repeated cerebral anemia; stellate ganglia excised; 14th successive occlusion. Dissociation of two peaks very well marked. Level of pressure between occlusions extremely low (20 mm. Hg. above base line).

C. Cat 53: Repeated cerebral anemia; adrenal glands ligated; 7th successive occlusion, obtained just before collapse. Drum revolving at same rate as above. Time of this reaction, 2 minutes. Control reaction in this animal, when fresh and intact had occupied 3½ minutes. Abruptness of initial rise and final fall characteristic of records after ligation of the adrenals.

identical to that in intact animals: ether was reduced, a corneal reflex elicited, and successive occlusion of the arteries done as soon as recovery from the last preceding occlusion had occurred. The cats differed somewhat in the rapidity with which the effect of the ligation of the adrenals appeared in the anemic blood pressure curve. In two cats, 52 and 56, the first occlusion following ligation showed little difference, and certainly no curtailment when compared with the control curve. In cat 52 the level of maximal pressure was maintained 2 full minutes longer than in the control. However, in the other four cats, the curves obtained following ligation of the glands immediately presented a marked contrast as compared with the normal occlusion and with the records obtained under repeated occlusion in the control series of intact animals. The characteristic feature of this change appeared at once and was retained until failure of the animal. This was an absolute halving of the occlusion time, and the retention, either of a reduced double curve, or of a single vigorous rise. In the two cats, 52 and 56 already referred to, this same reduction appeared somewhat later in the record. The occlusion time was not halved in cat 52 until the fifth occlusion following ligation of the glands; in cat 56, not until the fourth occlusion. In both these cats there also remained a distinct double rise in the pressure curve, which was not observable in the curves from the other animals. Autopsy showed no difference in the completeness of the ligation in these two animals.

In the cats in which the adrenals had been ligated the complete inability to restore the bulbar functions had to be faced in all cases before nine successive occlusions had been made. There were no exceptions to this early complete collapse in any of the cats observed. Two cats, 54 and 52 already mentioned, gave eight successive occlusions after ligation of the glands. Cat 53 gave seven; (fig. 5 C) cat 57, five; cat 56, otherwise so resistant to a change in its long occlusion periods and retention of normal contour, succumbed after only four occlusions. Only one occlusion was obtained from cat 55. Figured in hours of survival under this procedure, this meant a maximal survival of $2\frac{1}{2}$ hours, a minimal survival of 15 minutes. However, only two cats of the series failed within an hour of the ligation of the glands under successive occlusions. The average survival time was $1\frac{1}{2}$ hours.

Very few indications of the approaching collapse appeared in the record, the only index being perhaps the very low level of blood pressure between any two successive occlusions. This low level was established in all cases immediately after the spontaneous fall of pressure

closing the first occlusion that followed ligation of the adrenals. At this time pressure fell to 30 or 40 mm. Hg.—a level 20 to 30 mm. lower than in the intact animals at a corresponding time. In spite of the subsequent return of respiration and other bulbar activities, the pressure remained uniformly low. A return of the corneal reflex was obtained even at this reduced level. The level of blood pressure maintained between successive occlusions varied somewhat. On comparing the amount of recovery of blood pressure in a given animal after occlusion, and the number of occlusions obtainable, it was found that, at least in the extreme cases, a direct variation could be noted. The two very vigorous animals which gave eight reactions after ligation of the glands, cats 52 and 54, showed a recovery of pressure of 40 to 50 mm. during the period following release of the head arteries; whereas cats 55 (one occlusion) and 57 (five occlusions) never regained more than 10 mm. at the time of the return of respiration. Cats 53 (seven occlusions) and 56 (four occlusions) occupied a rather intermediate position, never showing an increase of more than 20 mm. pressure during recovery of bulbar function.

No change in the time needed for the return of medullary activity, as determined by the return of respiration and ocular reflexes, was noted in these animals. As in the control series of repeated occlusion in intact animals, this was not different after ligation of the adrenals from that obtained in the fresh animal. Periods of recovery of from 10 to 20 minutes were recorded, being fairly constant for the given individual.

No significant decrease in the anemic increment was observed, in cats 53 (seven occlusions) and 57 (five occlusions) where increments of 120 to 140 mm. were obtained just prior to failure. These were oddly enough the smooth curves recorded under early collapse. A much more pronounced decrease in the anemic increment was shown in the other animals in which more occlusions were obtained; in cat 52, (eight occlusions) the last occlusion recorded showed an increment of only 65 mm.

The contour of the curves obtained is of considerable interest. Cats 53 and 56 immediately showed a single rise occupying about half the original occlusion time (fig. 6), and this was a smooth unbroken curve. Though the reduction in time was just as manifest in all the other cats, the obliteration of the double nature of the curve was not so clearly marked. In these cats the characteristic contour of the anemic rise as seen in the fresh animal, merged gradually into the smooth curtailed curve following adrenal ligation. The changes most evident were the

greater abruptness of the initial rise while the depression of level due to vagus activity was apt to be increasingly delayed and tended to appear on the crest of the wave, somewhat similar to the effect described after section of the accelerators. The precipitous fall which occurs in these animals with ligated adrenals just about half as late as in intact cats then appears as soon as the point of maximal pressure is gained, namely, immediately after cessation of the vagus depression.

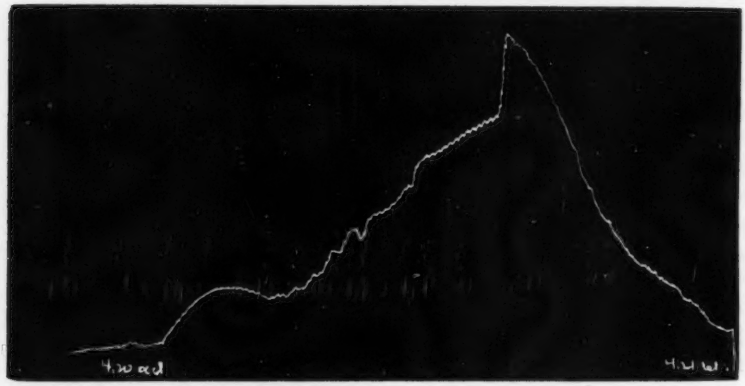

Fig. 6. Cerebral anemia following ligation of the adrenals. Cat. 53. Arterial occlusion immediately following tying off of the glands. Occlusion time, 1½ minutes. Time of control occlusion obtained from this animal, 3 minutes.

Effect of the adrenal glands on the anemic rise. The marked shortening of the anemic response eventually obtained in all the animals in which the adrenal glands had been ligated seems to isolate a further factor concerned in the production of the anemic rise. Apparently the great influence which the splanchnic system is able to exert on the level of blood pressure under the critical conditions of anemia, is due in part to the adjuvant activity of the adrenal glands. These experiments, therefore, bear on the discussion of the emergency relation of the glands, since apparently some involvement of the glands or some product of their activity must be conceded under the extreme condition of cerebral anemia. Furthermore, some clue as to the nature of the activity of the adrenals is given by inspection of the curves obtained.

Loss of pressure. There has been noted a close parallelism between the later curves obtained from all animals suffering repeated occlusion, whether intact or deprived of adrenals. A failure of blood pressure

(temporary or permanent) is recorded under both conditions within half the time normally occupied by the blood vascular response. When the animal is intact, this drop of pressure occurs in the seventh or eighth occlusion and in all subsequent curves of a given series. When the adrenals are ligated, it may be established immediately, although this is not necessarily the case. Under these conditions the halving of the response is recorded before four successive occlusions have been inflicted and is found in all occlusions which follow in these animals. This precocious loss of level in blood pressure can therefore be obtained either when the animal has been exposed to rapidly repeated cerebral anemia, or when the activity of the adrenal glands is completely abolished. Accordingly, the main factor in the production of this early failure of pressure seems concerned in all cases with the availability in the blood stream of some product of adrenal activity.

Restoration of level of blood pressure. An examination of the supplementary rise of blood pressure in the repeatedly occluded but intact cats in comparison with the permanent failure of pressure at half the normal occlusion time when the adrenals are ligated, leads to a consideration of the theories of emergency function of the adrenals. This secondary rise is most probably related to the presence of functional adrenals.

The secondary rise was interpreted in a preliminary report of these experiments as an indication of an increased liberation of adrenalin from the glands, and the constant interval prior to its appearance, as a latent period, relatively long, of adrenal secretion. The argument was advanced that these experiments offered evidence confirming Cannon's position on the increased secretion of adrenalin under emergency conditions. However, in view of the presumable consumption of the products of adrenal activity during cerebral anemia already discussed, the conception of the emergency liberation of adrenalin must be somewhat modified. The further discussion of any of the current hypotheses of the liberation of adrenalin must be deferred until further experimental evidence has been accumulated. Two definite statements, however, appear justified by the facts. In the first place, since curves of perfectly normal contour were obtainable in two animals after ligation of the adrenals, an increased liberation or secretion of adrenalin, one or both, is not necessary for the carrying out of the typical blood vascular response to anemia in the fresh animal.

That these results were due to experimental error can hardly be possible since there was seen in these animals a gradual and relatively slow

transition of the normal curve into the abbreviated response typical for animals with ligated adrenals. Such a gradual transition is also found in the intact repeatedly occluded animals. In the second place, from the premature failure of the vascular response, after the ligation of the adrenals, particularly in contrast to the secondary rise that is seen in the intact repeatedly occluded animals, the conclusion may be drawn that some product of adrenal activity must be available to make possible the continued action of sympathetic nerve on smooth muscle for any length of time.

Survival after adrenal ligation. In all the work reported on excision of the adrenal glands, sudden death has never been noted. However, when all adrenal tissue is excised, collapse and death follow, the interval of life varying in different animals. The earlier work on cats has been reviewed by Hultgren and Anderson (109), who particularly described the prelethal stage. Elliott (73) recorded the failure of blood pressure in addition to the loss of the pressor reaction in the moribund cat, and in a later paper he has summarized a series of tests given in these conditions, demonstrating a complete collapse of vascular tone. Gautrelet and Thomas, (110) later Hoskins, (111) have confirmed the depression of the sympathetic system on final collapse. Elliott records death with simultaneous extirpation under ether after 14 to 18 hours. Bazett (112) has recently succeeded in shortening this time considerably by decerebration, urethane anesthesia and sensory stimulation. In these animals the fall of blood pressure occurred within a few hours after the operation. Elliott (98), moreover, finds that the animal survives even if the adrenal tissue is separated from the splanchnics. He concludes therefore that, whereas the increase of adrenalin in the blood stream under splanchnic stimulation is not necessary to life, the animal depends for its existence on the continual slow secretion of adrenalin from the medullary cells. Elliott argues that this continual slow secretion is independent of nervous impulses. Stewart and Rogoff (113), (114), however, are unable to demonstrate any appreciable adrenalin output under these conditions.

It seems that the repetition of the extreme procedure of occlusion is able to hasten the onset of complete failure most surprisingly. In the extreme conditions of these experiments Bazett's (97) already curtailed time of survival after ligation of the adrenals is thus further shortened by 6 or 8 hours. The only demonstrable factor in the failure under these conditions is the inability of the sympathetic nervous mechanism to maintain the normal state of the musculature of the blood vessels

after complete exhaustion of the reserve of adrenalin in the blood. The necessity for the presence of adrenalin or of some other product of adrenal activity in the blood for the maintenance of vasomotor tone, as asserted by Elliott, seems again confirmed. The failure of blood pressure alone seems able to carry with it the failure of all the other functions.

From the evidence, the relative degree of constriction of the vessel walls seems, to a considerable extent, a function of the amount of some adrenal product in the circulation. The loss of this product seems to mean complete failure; blood pressure stays only a few millimeters above base line when the available supply is low, but an increased liberation, or possibly even a redistribution, may give any degree of tonic contraction of the vascular muscles, reaching to maximum constriction, the entire reaction perhaps depending on conditions at the myo-neural junction.

III. Relation of the splanchnic sympathetic system to the central nervous system. The central relations of the sympathetic system have been tenaciously disputed, and cannot be entered into at length. On the one hand, there has been the view defending its relative independence from the cerebro-spinal axis, originally advanced by Bichat (115), and supported extensively by Volkmann (116). Goltz in his latest work with Ewald (117) subscribed to this view, in his assertion that the sympathetic peripheral ganglia could maintain normal vascular tone, and mediate reflexes quite independently of the central nervous system.

However, the theory that the nervous outflow is essentially dependent for its activity on cells of central, and particularly bulbar origin, has always enrolled some powerful supporters. Two of Goltz's contemporaries, Eckhard (118) and Mayer (119) have defended this conception. Recently Gaskell (120) and Sherrington (121) and still later Ranson, (122), also endorsed it.

Two points in the evidence on cerebral anemia will briefly cover the relation of the medullary cells to the peripheral response. First, the comparison of the splanchnic response with the other peripheral responses, and particularly the skeletal responses as controlled by the medullary or higher cells, under the different functional conditions of the nervous levels in these experiments; second, the behavior of the blood pressure responses under recovery from various spinal lesions.

Comparison of splanchnic response with other peripheral responses. The following table gives the various stages which can be distinctly separated when different functional levels control the animal's reac-

tion. The rough average of the level of blood pressure maintained is given for each period. The exact correspondence of the involvement of the splanchnic system and the degree of functional activity within the medullary centers is indeed striking, especially in view of the potency of the splanchnic system in maintaining blood pressure. It appears from this tabulation that the splanchnic system behaves exactly as do the respiratory, skeletal and ocular responses. When the skeletal responses dependent on the higher levels are in abeyance, the vasomotor responses of the splanchnic system are also absent. At this time, moreover, that is, during the depression between occlusions, the heart rate shows no appreciable change. The level of blood pressure maintained is that shown by Couty (16), Mayer (14), Pike (26) and Langley (27) to be that held as long as the spinal cord itself remains intact. Additional evidence that the depression of functional activity is due to a complete interruption of conduction in the spinal cord, and not to so-called spinal shock, is brought out by the behavior of the animal in passing through these various stages. The bearing of the validity of the shock hypothesis for any conception of the functional organization of the nervous system has been discussed by Pike (123). A shorter statement of this relation is found in Yates's paper (36).

Comparison of somatic and ocular responses with vascular responses

Control of the animal's responses by various nervous levels	*Average level of blood pressure*
1. Normal intact animal: responds as a whole, pupils narrow, corneal reflex, respiration normal.....	120 mm.
2. Head subjected to anemia: struggles—responses under control of stimulated area (head) skeletal convulsions, respiratory spasms, corneal reflex lost.	180–200 mm.
3. Head functionally dead,—animal spinal:—responses under control of spinal cord only no corneal reflex, pupils widely dilated. No spontaneous respiration. No skeletal reflexes elicitable.....	50–70 mm.
4. Recovery of head centers: gradual return of responses controlled by head area, pupils narrowing—no corneal reflex—spontaneous respiration returns after pressure has risen somewhat, but still sporadic. Skeletal reflexes elicitable in part.....	70–90 mm.
5. Recovery completed; animal responds as a whole. Pupils narrow, corneal reflex, respiration reëstablished; functions coördinately, skeletal reflexes present.....	120 mm.

When a significant lesion in the splanchnic system has been inflicted by the section of these nerves just before entrance into the coeliac

ganglion and the cerebral circulation shut off, no anemic increment is obtainable. However, all other evidences of bulbar activity are present. A vigorous corneal reflex is obtained prior to occlusion, and when the clamps are adjusted, even though the level of pressure may remain more undisturbed than that obtained under many minor manipulations, the other symptoms of the asphyxial response are shown in full vigor. There are marked respiratory spasms, skeletal convulsions, changes in the pupils, etc.

In marked contrast to such a picture are the effects when, from some physiological disturbance, the medulla itself is thrown out of activity. Here the effects of the interruption of functional continuity are opposed to the effect of anatomical separation. Such a condition is present while the animal is still profoundly under the effect of an occlusion that has just been done, or even during recovery from occlusion, when the functions of the brain stem are not yet fully established. If, under such conditions, the animal is subjected to a renewed occlusion, no response at all can be aroused. Generalized asphyxia, inflicted by clamping the trachea and thus acting directly at the periphery is, in this condition, also impotent to produce any effect. Under this general depression there are no skeletal convulsions, no respiratory gasps, and pressure changes are extremely slight, 5 or 10 mm. The heart just quietly runs down. The condition of the eyes remains unchanged throughout.

All the evidence of these experiments therefore would argue not only for a normal release of the rise of blood pressure through the sympathetic outflow, but also for a complete dependence of the activation of the response on the integrity of the brain stem, and the maintenance of the conditions of conductivity within it. The response transmitted by the sympathetic system is functionally exactly on a par with all the other physiological responses. When respiratory movements, eye movements and skeletal reflexes are obtainable, the changes of blood pressure can also be elicited.

The anatomical relations of the splanchnic outflow in its bearing on recovery after section of the spinal cord. The complete dependence on the brain rather than on the spinal cord is well illustrated by experiments on recovery of blood pressure from high section of the spinal cord. Goltz, (17), (18) and later Goltz and Ewald (117) sectioned the cord of dogs in the midthoracic region and found normal blood pressure responses to subsist. These were attributed entirely to the controlling influence of the cord over the sympathetic system. These experiments have been repeated by later investigators, notably Sher-

rington (121), (124). In view of the very high level of the cord in which a lesion must fall before it can definitely intercept the connections between the medulla and the splanchnic effectors, there is a possibility that the agency concerned in this recovery is none other than the splanchnic constrictors still in functional continuity with the brain stem.

Four early experiments were carried out with Doctor Pike's coöperation in which a transection in the upper levels of the spinal cord was done aseptically, the animal allowed to recover and then the anemic response tested. In two cats the transection was done at the level of the 2nd thoracic. One animal died within 24 hours before the blood pressure could be tested; the other lived 5 days and was then subjected to the test of occlusion. Blood pressure, however, was very low, the bulbar responses failed immediately and no rise was elicitable. Cat 63, however, in which section at the 3rd thoracic was made, recovered fully and when tested a week later showed a level of blood pressure of 120 mm. and an anemic increment of 50 mm. in the first occlusion. Cat 64 with a section at the 6th thoracic was tested 2 days later. Control level of blood pressure was 80 mm. The anemic increment of the first occlusion was 45 mm.

This problem was subsequently taken up by Miss Yates under Doctor Pike's direction, and has been reported on in detail in an earlier issue of this Journal. Miss Yates (36) found that when one or two segments of the thoracic region only are left intact there is a recovery of blood pressure to an adequate level, and vigorous anemic or asphyxial response is readily elicitable. This recovery is attributable to those medullary cells still in connection with the peripheral splanchnic neurones.

It was on the evidence of Goltz's experiments that Langley applied the name "autonomic" to those peripheral mechanisms supplied by ganglionic connection outside the nervous system which he thought could function independently of the brain. The physiological evidence that is now accumulating would gravely discredit this autonomy, and would tend to place the sympathetic responses in the same category as all others. This is of particular importance in connection with the late appearance of the adrenal effect in cerebral occlusion, even after the reflexes are no longer elicitable. However late its appearance, and however independent of any parallel nervous activity, this effect certainly cannot be aroused unless the splanchnic fibers themselves have been previously stimulated. When the splanchnic fibers are no longer excitable because of an anatomical lesion the adrenal effect never appears.

This relationship is of particular interest with respect to the appearance of Traube-Hering waves. These have been noticed by all observers in the downward course of the final fall of blood pressure in the anemic response. Whether nervous centers are no longer excitable to sensory stimulation and whether or not the output of adrenalin is a factor concerned, remains to be tested. It must, however, be borne in mind that such a condition as this where Traube-Hering waves have been elicited, is preceded by an intense activity of the splanchnic outflow as stimulated by the medullary cells.

In the disturbance of the internal medium which the excessive concentration of carbon dioxide in the occluded cerebral vessels brings with it, the increased rate of flow is carried out and maintained by the vascular musculature, and some product of adrenal activity probably makes possible the maintenance of the increased impetus given the blood flow through such a prolonged period of time. However, it is only by virtue of the neurones within the central nervous system that the response is initiated, that it is regulated by changes in the cardiac musculature, and finally that the response is carried out as an integrated whole. The retention of a constant tension of carbon dioxide in the blood by means of an adaptive blood vascular reaction, is therefore mediated in the mammal through its higher central nervous organization, particularly the cells within the medulla oblongata.

To Prof. F. H. Pike the writer is greatly indebted for suggestions, advice and criticism, extended throughout the research.

CONCLUSIONS

1. The nerves of the heart are not essential either for the activation or for the persistence of the characteristic pressor phenomena of the anemic rise.

2. In the early stages of cerebral occlusion the cardiac innervation functions as a check on the rapid rise of blood pressure. In this moderating action, accelerators as well as vagi are involved, since on excision of the stellate ganglia, the vagi alone are unable to prevent an abrupt and steep rise of pressure.

3. The activation and maintenance of the vascular response under cerebral occlusion is controlled essentially by the splanchnic nerves.

4. Differential section in various regions of the splanchnic outflow influences the level of the arterial blood pressure. The extent to which the pressure falls on section is an approximate index of the degree to which the anemic rise will be compromised by the lesion.

5. It is impossible to influence the vascular response to anemia by indiscriminate sections within the splanchnic outflow. In order definitely to abolish the response, it is necessary to section either sufficiently far out in the periphery, or sufficiently high up in the spinal cord to interrupt completely the continuity between the medulla and the coeliac ganglion.

6. The level at which the fibers of the splanchnic system leave the spinal cord varies in different individuals. The greatest number of fibers leave the cord in the lower thoracic, especially in the region of the 6th to 8th thoracic. However, constrictor fibers to the splanchnic nerves leave the cord throughout the higher levels of the thoracic cord. In certain individuals, fibers leaving as high as the 2nd and 3rd thoracic will maintain an appreciable level of blood pressure and activate a significant anemic response.

7. Cerebral occlusion, carried out in repeated succession, is borne indefinitely (as many as 18 times) in intact animals. The occlusion time is in no way curtailed and the anemic increment of blood pressure only slightly diminished.

8. The curve of the anemic rise under repeated cerebral occlusion becomes dissociated into two distinct parts after eight or ten successive occlusions have been inflicted.

9. The long-continued maintenance of blood pressure at an extremely high level, characteristic of the anemic rise, is no longer possible after any gross interference with the supply of some product of adrenal activity.

10. An increased liberation of adrenalin under extreme splanchnic stimulation cannot be demonstrated as necessary for the characteristic contour of the anemic rise. This appears dependent on the amount of circulating adrenalin.

11. An increased availability of some product of adrenal activity appears demonstrable in intact animals under extreme splanchnic stimulation, after eight or ten successive occlusions have been inflicted.

12. Survival after ligation of the adrenal glands may be reduced to 1 or 2 hours, when the animal is subjected to successive repeated cerebral occlusions. A complete failure of vasomotor tone seems demonstrable in these animals.

13. The response of the splanchnic nerves is dependent for its release on conditions of functional activity within the brain stem.

14. The vasomotor responses initiated by the splanchnic nerves of the sympathetic nervous system are comparable with skeletal responses

dependent on the higher nervous levels, in respect to their complete dependence on these levels of the central nervous axis.

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THE RÔLE OF THE SODIUM AND THE CARBONATE IONS AND OF THE CHANGE IN THE SODIUM-CALCIUM RATIO IN THE CONTRACTION OF THE ISOLATED DUODENAL SEGMENT OF THE ALBINO RAT

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Previous studies have shown that the isolated duodenal segment of the adult, male, unexcited albino rat when suspended in oxygenated Tyrode's solution at body temperature contracts when small amounts (0.1 to 0.4 cc.) of tenth molecular sodium carbonate solution are added to the surrounding liquid (1), (2).

Two questions that naturally arise as a result of this reaction are: What is the intestinal mechanism stimulated by the sodium carbonate? and—What component of, or condition occasioned by the addition of sodium carbonate to, the surrounding Tyrode's solution furnishes the stimulus to contraction?

In a previous publication it has been shown that the contraction in question results from a stimulation of the musculature by those nerve elements normally concerned in contraction (3). This neural stimulation is set into activity by one or more of the components of, or conditions occasioned by the addition of the sodium carbonate to, the Tyrode's solution bathing the segment.

This addition causes an increase in the concentration of the sodium ions and of the carbonate ions. There is also an increase in the sodium-calcium ratio and in the hydroxyl ion concentration. We will consider the first three of these possibilities in this report and leave the last for the succeeding paper.

In general the procedure in these experiments was the same as that already described (2), (3).

Now if the shortening of the segment is due to a stimulation due to the increase in the sodium ions, the addition to the solution of an amount of these ions in the form of sodium chloride equivalent to the amount of sodium ions added as sodium carbonate, should produce the same height of contraction as results from the latter stimulus.

If the contraction following the sodium carbonate application is a result of an increase in the sodium-calcium ratio, there is no reason why the equivalent increase of this ratio should not produce the same result as when the ratio is increased by sodium carbonate. This possibility is to be considered since in the opinion of Loeb (4) changes in the concentrations of antagonistic ions or salts are the means by which stimulations are brought about, and this opinion is supported not only by his own studies but also by those of Osterhout (5), Lillie (6), Benda (7) and others.

Since, however, the addition of 0.4 cc. of a tenth molecular solution of sodium carbonate to the 4.0 cc. of Tyrode's solution occasionally gives rise to a perceptible precipitation of calcium salt, the use of that salt as a standardizing reagent in these tests is obviously inadvisable. Instead of sodium carbonate, then, we have used sodium bicarbonate as the standardizing substance, for when 0.4 cc. of a fifth molecular solution of this compound is added to 4.0 cc. of Tyrode's solution no visible evidence of calcium precipitation occurs during the test, and a characteristic shortening of the segment is obtained, as already shown by Young (8).

With these facts in mind the effect of 0.4 cc. of a fifth molecular solution of sodium chloride on the duodenal segment of the albino rat standardized with 0.4 cc. of a fifth molecular solution of sodium bicarbonate was investigated according to the procedure employed in the earlier studies (11), (3).

The results of such a test are shown in the accompanying tracing.

It is evident that the addition of an amount of sodium in the form of sodium chloride to the Tyrode's solution equal to that added in the form of sodium bicarbonate failed to produce the characteristic contraction brought about by the latter compound. This fact allows us to conclude that neither the increased sodium ions as such, nor the increased sodium-calcium ratio is the significant factor in causing the contraction.

The objection may be raised that the addition of this amount of sodium chloride to the 4 cc. of Tyrode's solution is too small to seriously affect the sodium-calcium ratio. If we grant the objection we can nevertheless counter with the observation that the same or even a lesser increase in the sodium-calcium ratio when brought about by sodium in the form of sodium bicarbonate causes a contraction of the segment and our conclusion is still justified.

For a substance to act as a stimulant to contraction it must reach

the mechanism on which it is to act. In other words, the tissue cells must be permeable to the effective agent. The importance of the permeability of the cell has been recognized since the time of Asclepiades (10) who based his system of medicine on the belief that health and disease depend more or less on the relation existing between the

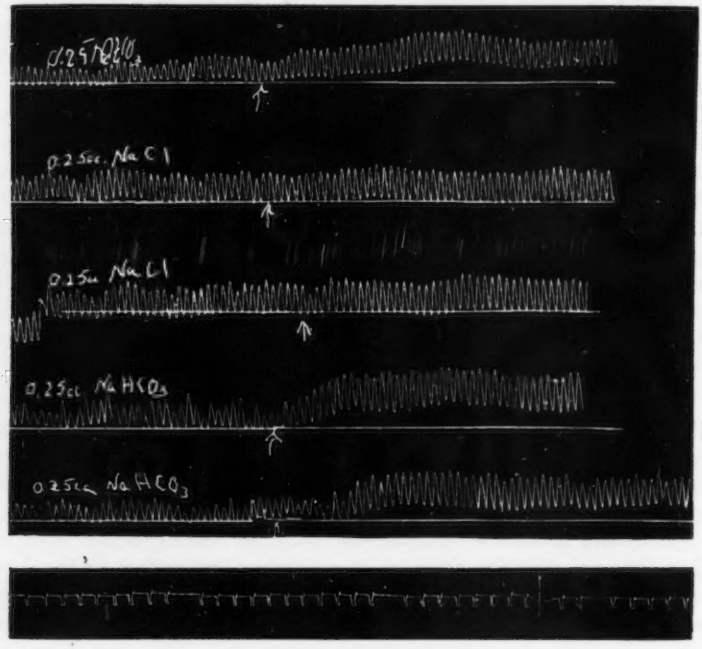

Fig. 1. The effect of fifth molecular sodium chloride solution on the isolated duodenal segment of the albino rat. The first two tracings show the effects on the segment of 0.4 cc. of a fifth molecular solution of sodium bicarbonate. The third and fourth tracings were made when there had been added 0.4 cc. of a fifth molecular solution of sodium chloride. The fifth tracing was made with sodium bicarbonate.

molecules and the pores through which they must pass. The studies of Loeb (11) and Osterhout (12) have quite definitely shown that permeability is favorably influenced by sodium ions and unfavorably by calcium ions. It may be that the increase in the sodium ions as such or through the increase in the sodium-calcium ratio facilitates, as it were, the admission of the exciting ions to the stimulated mechanism. Thus the sodium ions can be considered as participating

in the reaction, but they can not in any way be considered as the agents setting the neural mechanism into that activity which results in a shortening of the duodenal segment. This belief is directly opposed to that of Loeb (13) with respect to the hydroxyl ion effect.

As far as the carbonate ions are concerned, they can hardly be considered as an important determinant of the response since one of us has shown that the duodenal segment reacts to all appearances to sodium hydroxide just as it does to sodium carbonate (14). Although the addition of sodium hydroxide solution to Tyrode's solution is presumably accompanied by the reaction

$$NaOH + NaHCO_3 = Na_2CO_3 + H_2O$$

this can have but little if any effect upon the concentration of the carbonate ions when there is taken into consideration the relative ionization of the dissociation products of sodium carbonate.

CONCLUSION

The contraction of the isolated duodenal segment of the albino rat which follows the addition of tenth molecular carbonate solution to the oxygenated Tyrode's solution in which the segment is suspended, is due neither to the increase in the sodium ions, nor in the sodium-calcium ratio, nor in the carbonate ions. It is true that the increase in the sodium ions may participate in the effect by increasing the permeability of the tissue for the agent initiating the reaction, but this increase in permeability can not be considered as the primary cause of the contraction.

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THE RÔLE OF THE CHANGE IN HYDROGEN-ION CONCENTRATION IN THE MOTOR ACTIVITIES OF THE SMALL INTESTINE

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In the preceding paper (1) it was shown that the contraction of the isolated intestinal segment of the albino rat suspended in oxygenated Tyrode's solution at body temperature on the addition of fifth molecular sodium bicarbonate to the surrounding fluid, is not due to the increase in either the sodium ions, the sodium-calcium ratio or the carbonate ions.

In this paper there will be presented evidence for the view that the effective agent for the observed contraction is the increase in the hydroxyl-ion concentration of the fluid in which the segment is suspended following the addition of the alkaline compound used.

In view of this result and other correlated data the probable relation between the changes in hydrogen-ion concentration of the material coming in contact with the intestine and intestinal movements will be discussed.

In order to determine whether or not the observed contraction of the isolated intestinal segment is due to the increase in hydroxyl-ion concentration of the Tyrode's solutions on the addition of tenth molecular sodium carbonate there was prepared a solution of sodium hydroxide which would give the same hydroxyl-ion concentration when added in the same amount to equal quantities (4 cc.) of Tyrode's solution as is given by the tenth molecular sodium carbonate solution. Using the indicator method it was found that the addition of 0.2 cc. of a solution of sodium hydroxide of approximately twentieth molecular concentration to the 4 cc. of Tyrode's solution gave a pH between 9.4 and 9.8. The addition of 0.2 cc. of a tenth molecular solution of sodium carbonate to 4 cc. of Tyrode's solution gave the same pH. A comparison of the

[1] Much of the experimentation reported in this paper was carried on by Mr. J. E. Nowrey, Jr., to whom the author wishes to express his thanks.

two resultant colored solutions in the Duboscq colorimeter showed an agreement in color depth that was satisfactory.

Intestinal segments were prepared as described in a previous publication (2) and their responses to the addition of equal amounts of these two solutions to equal amounts of Tyrode's solution in which they were suspended were recorded as usual.

It was found that practically the same degree of contraction was obtained with the sodium hydroxide solution as with the sodium carbonate solution. This is shown in the accompanying tracing. It allows of no other conclusion than that *the stimulus to contraction is the increase in the hydroxyl-ion concentration of the liquid in which the segment is suspended.*

This result is also a confirmation of the findings of the preceding (1) paper, because on the one hand the concentration of the sodium ions in the sodium hydroxide solution used was less than a fourth of that of the sodium carbonate solution; and on the other, the carbonate-ion concentrations were in no sense comparable.

This result may also serve in part, at least, to explain on the basis of the change in hydroxyl-ion concentration the stimulating effect of various alkaline salts on intestinal contraction observed by Salant, Mitchell and Schwarze (3), Salant and Schwartze (4), Alvarez (5), Starkenstein (6) and others.

Before discussing the indications of the foregoing observations a brief review of the studies leading up to the ideas about to be expressed is not out of place.

It was observed by Hatai and Hammett (7) that the isolated duodenal segment of the albino rat, when suspended in oxygenated Tyrode's solution at body temperature, responds by a contraction when small amounts of tenth molecular sodium carbonate solution are added to the liquid surrounding the segment. It was found that this reaction was reversed, i.e., that a relaxation occurred *a*, when the animal had been excited or angered previous to the removal of the segment for testing; *b*, when the splanchnics were electrically stimulated in the dead animal before removal of the segment; and *c*, during periods of physiological or emotional instability such as occur in young animals or menstruating females. It was also noticed that approximately equal degrees of contraction followed the application of equal amounts of the carbonate solution to segments from the normal, adult, unexcited male rat. Further investigations established the fact that segments from such animals could serve as accurate testing material for the quantitative

comparison of tissue extracts and other substances within reasonable limits when sodium carbonate is used as the standardizing reagent (2). These observations gave a basis for testing the intestinal contracting ability of thyroid extracts from the glands of rats of different ages (8) and the action of thyroxin on the isolated intestinal segment (9). The next study was devoted to a determination of the intestinal mech-

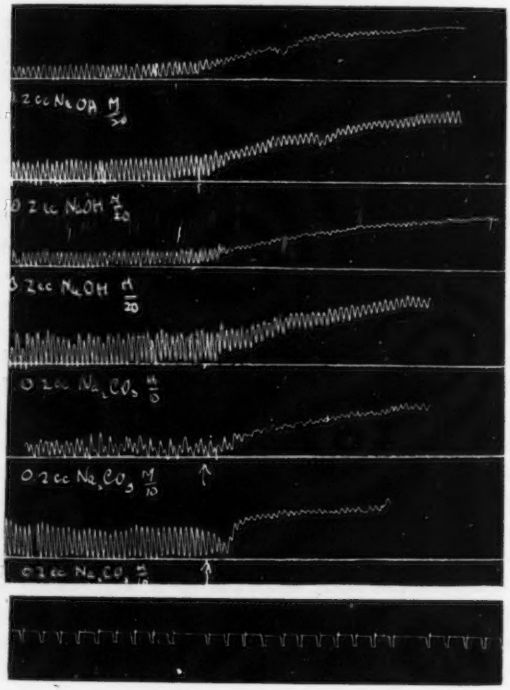

Fig. 1. Effect of equal H-ion concentrations from NaOH and from Na_2CO_3 on the degree of contraction of the isolated intestinal segment. Time in 5 seconds.

anism primarily stimulated by the carbonate (10). It was found that the neural mechanism is primarily involved in the phenomena recorded. This led us to realize that a determination of the agent or condition effective in producing the contraction might throw some light upon the nature of the factors directly regulating or controlling intestinal movements. The preceding paper (1) and the evidence presented in this

one have shown that neither the increase in the sodium ions, nor the sodium-calcium ratio, nor the carbonate-ion concentration is the effective agent, but that the stimulus to contraction is the increased hydroxyl-ion concentration derived from the dissociation of the added sodium carbonate.

We are at once confronted with the question as to how far we are justified in utilizing the findings of these experiments *in vitro* for an interpretation of the probable control or influence of changes in hydrogen-ion concentration on the motor activities of the intestine. The direct transfer of these observations must be made with caution and with reservations explicit or implied.

Although Cannon (11), Gaskell (12), Alvarez (13) and others have recognized the probability that the motor activities of the intestinal musculature are modified by chemical factors, nevertheless the interpretations of these phenomena, with the exception of the latter investigator, have been almost entirely based on the idea of a predominantly neural control as developed from the studies of Bayliss and Starling (14), (15), Magnus (16) and others. Nevertheless it is undeniable that the initiation of every stimulus which is carried to the muscle tissue by the nerve fibers is, in the last analysis, due to a chemical change. That the ultimate explanation will be based on energy changes is obvious and has been recently emphasized for biological processes by J. Traube (17).

It is not necessary to go into any detailed exposition of the proved and probable chemical sources of stimuli produced by the organism affecting either directly or indirectly the motor activities of the intestine. With no intention of minimizing the importance of their rôle, it is felt that the studies of Henderson (18), Michaelis (19), Loeb (20), Moore (21), Clark (22), Lillie (23), Osterhout (24) and others on the exquisite sensitivity of living cells to change in hydrogen-ion concentration give ample evidence of the predominant part which this type of chemical change plays in the phenomena of stimulation in general and of nerve stimulation in particular.

We have shown in this and previous studies that the intestinal segment shortens or contracts when an increase in the pH or hydroxyl-ion concentration occurs in the solution with which it is in contact. It has also been shown that this effect is produced by transmission of the stimulation through the neural mechanism of the segment. That other compounds which on dissociation in solution give rise to an alkaline reaction produce the same effect has been alluded to before.

On the other hand, Young (25) has definitely shown that an excess of hydrogen ions produces just the opposite effect, namely, a relaxation, when added to the solution in which the segment is suspended. The experiments of Alvarez (26) support these observations in their general aspect.

It is thus evident that changes in the hydrogen-ion concentration of the medium with which the intestine comes in contact have a marked influence upon its motor activities; an excess of hydroxyl-ions causing a contraction, an excess of hydrogen ions a relaxation.

That changes in the hydrogen-ion concentration of the intestinal contents are bound to occur is evident from the observations of Busch (27), Auerbach and Pick (28), McClendon and his collaborators (29) and others that the succus entericus is normally alkaline in reaction, and the finding of Foa (20) that the pancreatic juice is similarly constituted, when correlated with the observations of Moore and Bergin (31) and McClendon and his collaborators (32) that the reaction of the intestinal contents on removal after eating is normally acid. In addition there is the well-known fact of the passage of the hydrochloric acid of the gastric juice into the duodenum during the process of digestion. It is therefore certain that there takes place in the intestine a continuous swinging to and fro between alkalinity and acidity of the reaction of the material coming in contact with the intestinal wall.

During this play back and forth between these oppositely acting agents of stimulation there must arise innumerable opportunities for these changes to be transmitted by diffusion into the intestinal wall and into contact with the neural mechanisms concerned in the motor activity and there set into play now one, now the other type of response, according to the specific mechanism stimulated. That such may conceivably occur appears from the observations of Loeb and Wasteneys (33) that weak alkalies, and of Crozier (34) that weak acids penetrate the cellular membranes with extraordinary facility, and the findings of Langley (35) that it is the nature of the nerve endings and not the impulses carried by the nerves that determine the nature of the response.

We do not wish to be understood as holding that the stimulation to contraction or to relaxation is due to a direct contact of an excess of hydroxyl ions or of hydrogen ions derived immediately from the changes in reaction of the intestinal contents with the neural elements directing the motor activities of the intestine. While this may be so, it appears more probable that the penetration of the cell layers immediately in contact with the intestinal contents by varying hydroxyl- or hydrogen-

ion concentrations which set up their characteristic effects, is followed by a transmission of these effects through a local and generally limited zone until neutralized by meeting zones of opposite reaction. In this spread of the effect primarily induced by changes in hydrogen-ion concentration of the intestinal contents in contact with any given spot of the intestine at any given moment and which changes from moment to moment, there is encountered a portion of the neural mechanism of the intestine and the appropriate response is elicited depending upon the nature of the stimulus. Further analysis will probably reveal that this spreading effect is of the nature of physico-chemical changes in surface charges or potential following changes in ionization.

Inasmuch as little if anything is accurately known of the precise rôle of the neural supply to the intestinal musculature in the various motor activities exhibited in katastalsis, anastalsis, segmentation and the myenteric reflex it would be wasted speculation to attempt an analysis of the specific rôle of the changes in hydrogen-ion concentration in these phenomena.

Nevertheless it may be assumed from what is known of the general nature of the response elicited by stimuli coming in over the vagal or splanchnic pathways and the known effect of hydrogen-ion or hydroxyl-ion excess on intestinal movement, that the nerve endings of the vagal mechanism are affected by a predominant hydroxyl-ion concentration while the splanchnic endings are affected by a predominant hydrogen-ion concentration. This assumption, however, does not facilitate, in the present state of our knowledge, an interpretation of the phenomena of specific intestinal movements. Until information is to be had of the finer details of the neural direction of the intestinal motor activities, and until the changes in reaction of the fluids in their passage through the intestinal wall and their effect upon the protoplasm of the intestinal cells are known, any application of the above assumption is precluded.

This lack of information, however, does not destroy the validity of the idea that changes in the hydrogen- and hydroxyl-ion concentration of the material coming in contact with the intestine are important participants in the regulation and control of the intestinal motor activities.

We are not to be understood as taking the stand that the motor activities of the intestine are solely dominated by the effects of a local stimulation of the intestinal neural mechanisms by changes taking place in the hydrogen-ion concentration of the intestinal contents. Nor do we hold that changes in the hydrogen-ion concentration at any one point in the system are the sole determinants of the nature of the response

elicited. For there is ample evidence that a strictly neurogenic influence, in the sense of a central participation, may at times supersede a local chemical influence. The opposite may also occur. But we believe that in the normal undisturbed organism during digestion the changes in the hydrogen-ion concentration of the intestinal contents determine the intestinal motor activities which are superintended and directed by the intestinal neural mechanisms.

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THE ELECTRICAL CONDUCTIVITY OF ANIMAL TISSUES UNDER NORMAL AND PATHOLOGICAL CONDITIONS

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The researches of Lillie (1), Loeb (2), Osterhout (3), Mathews (4), McClendon (5), Hill (6), Lucas (7) and of many other biophysicists and chemists (8) indicate that the functions of the cells of living organisms are related to electrical processes; that the living cell, whether it exists alone or as an element in a complex organism, possesses a certain store of potential energy which is manifested by variations in polarity and by action currents; that variations in the permeability of the living cell to the electrically charged elements of the fluid which surrounds it parallel variations in irritability in response to stimulation; that factors which suspend or abolish irritability also suspend or abolish alterations in permeability.

Every activity of living tissue is accompanied by electrical currents; and many activities are also initiated by electrical currents. In fact, the work of the investigators referred to above shows how strong is the tendency to consider that vital processes depend upon electric energy, by means of which also the protoplasm is renewed, and the whole mechanism is constructed.

In view of this trend of physiological conceptions, the electrical properties of living protoplasm become of vital interest. As this interest extends, the need of definite quantitative data increases. The laws which govern the action of electrical forces in inorganic systems are known exactly. It is possible to calculate exactly how much heat, or what chemical change, or how much work will result from the passage of a current of known strength through a known resistance during a definite period of time.

The two independent factors, current and resistance respectively, depend upon the amount of available electrical energy and the constitution of the system in which its conversion into heat, or chemical change, or other type of work is to be accomplished.

The action of electrical energy in protoplasm, although all the conditions are far more complicated than in inorganic substances, is governed by the same laws. In protoplasm, as in inorganic matter, electrical currents will always choose the path over the lowest available resistance; and in protoplasm, as in inorganic matter, the current pays toll to the friction offered by the system through which it passes.

The facts already established regarding bio-electric currents are sufficient to indicate the importance of further investigation, especially along certain lines. For example: What is the range of the electric conductance of living tissue? How does that range compare with that of other electrical conductors? Is the range of conductance the same for all types of tissue, and in each tissue does it remain constant under all conditions? Is the electric conductance of each tissue a factor in the production of the activities of the organism, to which a fairly constant value can be assigned?

These are questions which occur at once to the most casual student of bio-electric problems, and the fact that the literature offers no clear answer is sufficient reason for a detached study of this subject.

During recent years various investigators have applied measurements of electrical conductivity to the determination of variations in the permeability of protoplasm under varying conditions. In particular the work of Osterhout, Lillie and Loeb along these lines is too well known to be more than mentioned here.

Other investigators have used conductivity measurements as a means for estimating the volume of the corpuscles in blood, for determining the H-ion concentration in body fluids, for measuring the variations in the conductivity of muscle which result from contraction. But none of these investigators have attempted to determine the specific conductance of any tissue.

Early in this century Galeotti (9) carried out a limited number of experiments for the purpose of studying the changes which occur at death. He utilized the tissues of dogs, rabbits, guinea pigs, frogs and turtles, making measurements at successive intervals after the removal of the tissue from the animal. In general, he observed a rapid decrease in conductivity during the first few minutes, followed by a more gradual decrease, which in some instances lasted for several hours. After reaching the minimum, which he considered marked the death point of the tissue, the conductivity began to rise, gradually at first, and then very rapidly until a very high value was reached.

His values for the normal conductivity of certain rabbit tissues may be of interest as compared with those observed in the work to be described later (tables 1 and 2).

For the most part Galeotti worked with the firmer tissues, sections of which he introduced directly between platinum electrodes which were clamped in place after the application of a greater or less degree of pressure. He used only a few animals of each species and does not

TABLE 1

Specific conductivity of certain rabbit tissues as determined by Galeotti. (Expressed in reciprocal ohms)

TEMPERATURE	LIVER	HEART	MUSCLE	
			Longitudinal	Transverse
12°C.			00182	00058
18°C.	000268	000799		000804
	00090			
	00053			
24°C.	000269			000789

TABLE 2

Comparison of the specific conductivity of rabbit blood before and after coagulation as determined by Galeotti. (Expressed in reciprocal ohms)

TEMPERATURE	BEFORE COAGULATION	AFTER COAGULATION
38°C.	00569	00566
	00679	00674
	00773	00771
	00539	00526
	00631	00590

state that he measured more than one sample of each tissue from any one animal.

The work to be described below was the direct outcome of preliminary measurements made by G. B. Obear of the Case School of Applied Science in the fall of 1917. In spite of inadequate apparatus and a limited number of observations, Doctor Obear's results indicated such consistent relative values of the specific conductivities of certain tissues, especially the cerebrum and cerebellum, as to encourage a further research under better conditions. In the fall of 1918, therefore, the research was continued.

Apparatus. The apparatus employed in this research includes that devised by Dr. E. W. Washburn and developed for the market by Leeds and Northrup. It consists of the typical Wheatstone bridge made up of a Kohlrausch slide wire and a resistance box of Curtis coils, with a telephone connected across the ends of the slide wire. Suitable capacities for tuning the circuit and for balancing the capacity of the conductivity cell were inserted in the current. A high frequency (1,000 cycles) alternating current was obtained from a constant speed high frequency generator located in another room. All measurements were made with the cell partially immersed in a constant temperature bath. A Freas bath of 300 liters capacity was used, supplemented by a specially constructed cover to minimize fluctuations of temperature, to maintain a sufficiently humid atmosphere, and to insure maintenance of the leads and upper part of the conductivity cell at a uniform temperature.

On account of faulty construction of the glass parts, the automatic temperature regulator for this bath has never worked satisfactorily, but by hand regulation of the lights it has not been difficult to keep the temperature constant within 0.1°C.

Washburn's recommendations in regard to magnetic shielding, grounding, etc., have been carefully observed.

Figure 1 shows the arrangement of this apparatus as used in this research.

Electrodes. As the early part of this work was done at a time when it was a patriotic duty to conserve platinum, in the preliminary experiments all types of tissue were measured in the same set of electrodes, though it is obvious that this is far from an ideal mode of procedure.

Sections of each tissue were packed into small glass tubes of various sizes, each of which was accurately ground to insure uniform dimensions throughout. The tubes were packed with a sufficient excess of material to procure a slight projection from each end, and were placed between thin platinum electrodes, reinforced by brass backings. Sufficient pressure was applied to bring the electrodes flush with the ends of the tubes, when the electrodes were firmly clamped into place. Great pains were taken to avoid air spaces within the tubes and to insure uniform contact of the tissues with the electrodes. This was not difficult with the softer tissues, such as brain and liver, but with tougher tissues such as muscle and thyroid it was impossible to exclude considerable error from imperfect contact and other variations. The effect of these faults is plainly evident in the greater variation in the conductance values obtained for the latter tissues.

The tubes used for the measurement of the conductivity of the brain, the liver and voluntary and involuntary muscle, were approximately 5 mm. in diameter and 5 mm. in length, while those used in the measurement of the adrenals and the thyroid were of the same length with a diameter of approximately 2.5 mm. Special hard rubber containers were devised for the spinal cord (fig. 2).

The conductance capacities or cell constants of these tubes were determined by repeated measurements of their conductance when filled

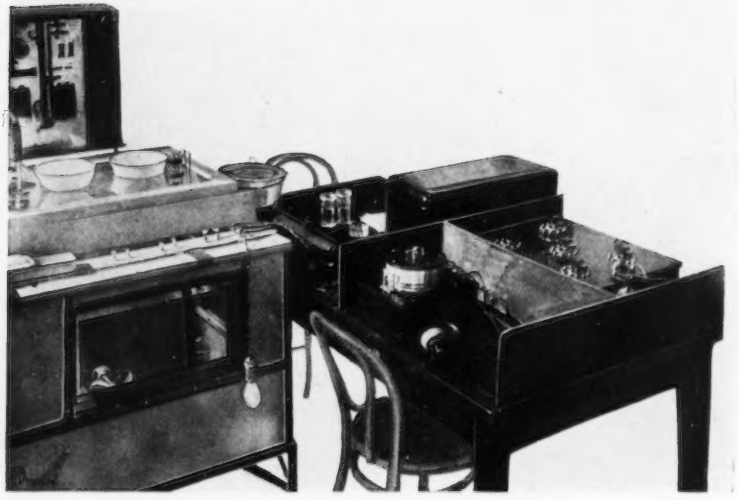

Fig. 1. Arrangement of apparatus for electrical conductivity research

with 0.01 NKCl, at the same temperature as that used for the tissue measurements.

In the later work larger tubes 1 cm. long $\times$ 1 cm. in diameter were used. These were only partially filled with tissue, and an upper electrode of the type shown in figure 3 was used. This electrode was 1 cm. in diameter and was pierced by slits. The tube was partially filled with closely packed material, and carefully placed in position on the lower electrode, after which the upper electrode was inserted within the tube and carried down until contact was made with the upper

surface of the tissue, care being taken, however, to avoid sufficient pressure to cause the extrusion of material through the slits. This upper electrode was then clamped in place and the distance between the two electrodes was accurately measured and recorded.

Every precaution was taken to avoid any undue pressure with the consequent reduction of the fluid content of the tissue and resultant

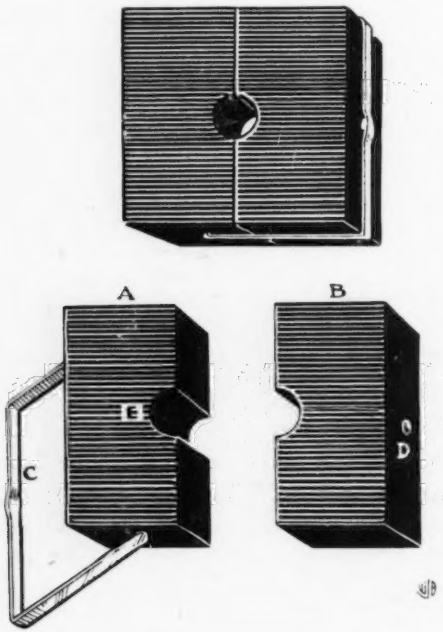

Fig. 2. Special container devised for making conductivity measurements of the spinal cord. *A* and *B* are blocks of hard rubber. The section of spinal cord is laid in the curved groove in one of these, the two pieces brought together and clamped by carrying the brass loop *C* over *D*.

lowering of the conductance, although this danger was lessened by the use of the pierced electrode. Various measurements of different types of tissue were made to determine the effect of varying the pressure, the results of which are illustrated by the groups of measurements shown in table 3. In each case the successive measurements were made upon the same sample of tissue, the distance between the electrodes,

and consequently the pressure, being changed between each two measurements. The results indicate that a greater error is to be feared from the application of too much pressure than from too light a contact.

The cell constants for the tubes used in the later experiments, as for those used in the earlier series, were determined by measurements with 0.01 NKCl with the electrodes at different distances apart, these

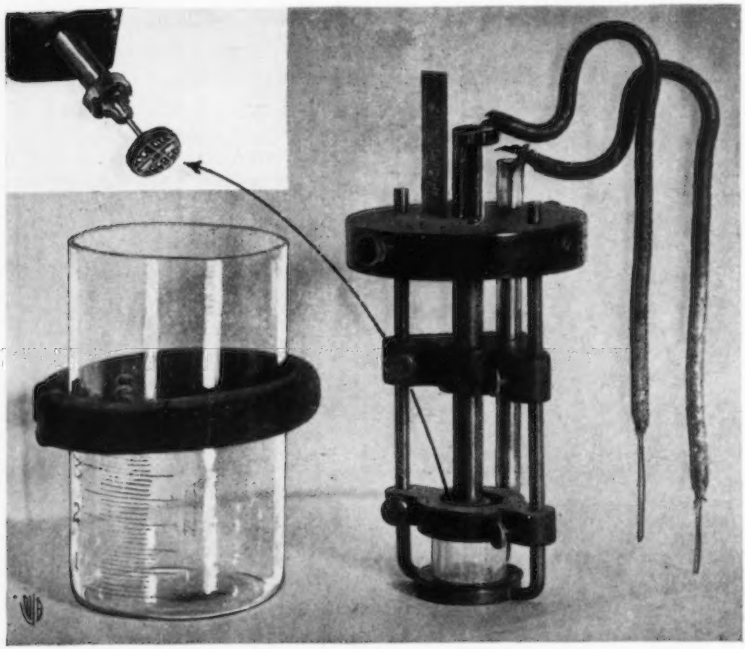

Fig. 3. Electrical conductivity cell and detailed drawing of upper electrode

results being plotted for convenient use in estimating the value of the tissue measurements.

The electrodes were carefully cleansed after each measurement and were replatinized at intervals with a very light coating of platinum black. The electrodes and holders were always placed in the bath long enough before use to insure the thorough warming up of the metal and glass parts, and during the insertion of tissue were exposed as little as possible to lower temperatures, and even then they were given

TABLE 3

Effect of variations in pressure upon the electrical conductivity of animal tissues

TISSUE	DISTANCE BETWEEN ELECTRODES	CONDUCTIVITY (EXPRESSED IN RECIPROCAL OHMS)
	cm	
Cerebellum		
Section 1	0 78	00133
	0 66	00129
	0 48	00114
Section 2	0.92	00148
	0 55	00128
Section 3	0.60	00140
	0.52	00136
	0 40	00123
Section 4	0.65	00115
	0.50	00108
	0 40	00105
Section 5	0 75	00121
	0 65	00116
	0 50	00116
Section 6	1.11	00158
	0.96	00151
	0.65	00128
Cerebrum		
Section 1	1.24	00183
	0.94	00167
	0.72	00159
Section 2	1.20	00183
	0.92	00170
	0 81	00169
Section 3	1.03	00172
	0.85	00164
	0.64	00145

an opportunity to reach the bath temperature before measurements were made. It was possible to exercise these precautions with but little loss of time by using two sets of electrodes and holders. All

measurements were either made at 39°C. or were corrected to that value from temperatures not over a degree removed from it.

Special points in technique. The object of this investigation being to determine the electric conductivity of animal tissues under conditions as nearly as possible identical with those existing in the living animal, every effort was made to keep the sections of each tissue always at a normal body temperature; to avoid any loss of moisture; and to measure each at the earliest possible moment after the death of the animal and the removal of the tissue from the body.

A series of measurements was made to determine the onset of post-mortem changes in the different tissues as evidenced by changes in the electric conductivity.

TABLE 4

Conductivity measurements made at varying periods after death to determine onset of post-mortem changes

(Conductivity expressed in reciprocal ohms)

	PERIOD AFTER DEATH							
	Immediate	½ hour	1 hour	1½ hours	2 hours	2½ hours	3 hours	4 hours
Cerebellum	00139	00140	00140	00148	00156	00178	00219	
Cerebrum	00175	00174	00178	00195	00201		00245	
Cerebrum	00181	00180	00185	00192	00202	00224	00241	
Liver	00075	00089	00099	00112	00117	00132	00156	00282
Liver	00081	00094	00097	00121	00165	00187	00236	
After ½ hour	00082							
Voluntary muscle	00486	00472	00484	00499	00498			00617*
Voluntary muscle	00194							00210*

*Following day.

We found that the conductivity of all tissues remained practically unchanged during the first hour after removal from the body; i.e., provided the section had been kept at a constant temperature in a humid atmosphere. The earliest post-mortem changes in conductivity were found in the liver and the brain; the latest in voluntary muscle. No significant change was noted in the brain in less than one hour after removal. In two instances liver changes began in approximately one-half hour after the death of the animal (table 4). The observation of these changes led to the adoption of an unvarying routine, in which the liver was always the first tissue to be removed, sectioned and measured.

As a corollary to the measurements made specifically for the purpose of determining the onset of post-mortem changes it may be pertinent to note here that observations of the electric conductivity of the liver after it had been tied off for four hours, but left in situ, to produce an effectual hepatectomy, showed the conductivity was increased far above that observed in any experiment in which immediate measurements of the conductivity of the liver were made.

Post-mortem change was not the only factor to be considered, however. It is obvious that animal tissues present no such ideally uniform material as is ordinarily subjected to conductivity measurements. Even if it were possible to secure sections of exact uniformity throughout, completely filling the space between the electrodes, an ideal that thus far has not been satisfactorily attained, it would still be necessary to consider variations in the structure of different parts of the same organ, as well as variations between individual animals.

It was obvious at once that at best we could expect only to establish the limits of variations for each tissue, and that if the results of these measurements were to be of any general physiological value, they must represent the findings in a large number of individuals.

For all these reasons it seemed inadvisable to employ any technique which would delay the prompt measurement of sections after their removal from the animal; or would prevent the examination of a large number of animals, even though such a technique might markedly increase the precision of any single measurement. The precision of a single measurement is of little significance if it is obtained after such a lapse of time as to permit a change from the conditions in the living animal. Also if the normal variation in the conductivity of the same tissue in different animals is several per cent, as one would expect to be the case, it becomes important to multiply the *number of normal measurements* as well as to try for a high degree of precision in single measurements.

On the other hand the absolute maintenance of constancy of method is imperative and no effort has been spared to insure this requisite. Uniformity of technique in the choice of animals, the method of killing, the nature of treatment, the lapse of time and conditions under which the tissue was kept between the killing of the animal and the actual measurement, in the method of measurement and in all other factors has been maintained just as far as possible.

Animals which showed any abnormal condition either before or during treatment or at autopsy were always rejected. The weight

and temperature of each animal were recorded. The animals received their last feeding the evening before they were used, as the measurements were always made during the morning; calculations, testing and adjusting of apparatus and so forth being done during the afternoon. The animals were killed by stunning with a blow on the head and the immediate severance of the veins and arteries.

The liver was at once removed, sectioned, packed in warm tubes and placed in the saturated atmosphere of the constant temperature bath for immediate measurement.

In the earlier experiments the block of liver tissue was removed and kept as nearly intact as possible. As it proved difficult to secure sufficient uniformity of filling of the tubes by this method, in the later work the liver substance was separated from the connective tissue and the resultant soft mass was carefully transferred to the tubes. Histologic examination shows that this treatment separates the lobules from the connective tissue, but as the size of the individual liver cell is 5 to 8 microns there is no reason to believe that more than a very small percentage of the liver cells themselves has been destroyed.

The other tissues were removed and sectioned in turn—always in the same order—and measured immediately. The time between the removal of a tissue until its measurement seldom exceeded 20 minutes and under no circumstances was the section allowed to cool perceptibly.

By careful training and close coöperation between principals and assistants, the technique was developed to such a point that it was possible to prepare and measure two sections each from the liver, cerebrum, cerebellum, spinal cord, voluntary muscle and involuntary muscle (heart), and usually one section only from the adrenals and from the thyroid, within 45 minutes after the death of the animal. In the later work, when in the majority of animals measurements were made of only the brain and the liver, it was possible to make measurements of a number of animals each day, and thus secure nearly uniform conditions in the different animals.

At the beginning of the research as many sections as possible were made of each type of tissue until a minimum percentage of variation for each was established.

Thereafter, whenever the measurements of different sections of the same organ failed to agree, the higher value was in general the one accepted, for the reason that all sources of error under the conditions employed in this work were such as would diminish the conductivity. Thus if the tubes were incompletely filled, that is, if they contained

air spaces; if there was imperfect contact with the electrodes; if the material protruded beyond the tubes used in the earlier work, so that the length of the section was greater than that of the containing tube; if too great pressure was exerted; if the temperature of the section was reduced below the normal—any of these conditions would produce a measurement below the true conductivity value.

Two readings of each section were made, an interval of one minute being allowed between the successive readings. The magnitude of the current used was varied according to the resistance of the individual section. The relation of the area of the cross section to the length of the section of each type of tissue was kept within the range that experience showed would give the most favorable minima on the telephone.

Range of electrical conductivity of normal rabbit tissues. The accompanying table (table 5) gives the average measurements of the electrical conductivity of tissues from 91 normal rabbits. In addition to these measurements preliminary studies made during the development of the technique, bring the total number of normal rabbits studied to over one hundred.

Besides the tissues included in the table, measurements have been made of the thyroid, the adrenals, the kidneys and the spleen. The variation in these measurements was so great that no averages for these tissues have been made and it remains to discover some method by which accurate measurements of these tissues and increased accuracy in the measurement of the spinal cord, of the heart and of voluntary muscle may be secured.

It will be noted that with the exception of the spinal cord, the order of magnitude of the conductivity values of the tissues included in table 6 never varied.

It was the initial plan to establish a normal range of the conductivity of the various tissues to be used as the basis of comparison for the tissues of all subsequently treated animals. Accident, however, showed the futility of this plan.

During the period in which groups I to III were measured, (November 1918 to February 1919) the animals had been kept in airy, cool quarters in the country, and provided with an open air run. In April they were removed to a typical animal room which, although well lighted and ventilated, was a great contrast to the former quarters. The effect upon the animals which were transferred is indicated by the measurements of group IV. The measurements of this group as well as of all

TABLE 5

Measurements of the electrical conductivity of normal rabbit tissues

GROUP		NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	SPINAL FLUID	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	BLOOD	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	BILE	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	HEART	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	VOLUNTARY MUSCLE	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	LUNG	AVERAGE DEVIATION PER CENT	NUMBER OF ANIMALS	SPINAL CORD	AVERAGE DEVIATION PER CENT
I.	11/14–11/22/18	7	00180	1 2	7	00155	2 6	9	00075	4 0																			7	00146	5 2
II.	12/14–12/21/18	6	00189	1 4	6	00164	0 8	3	00072	5 9																			6	00180	5 9
III.	2/12– 2/19/19	5	00192	1 6	6	00162	5 3	5	00074	2 2														4	00655	2 6			4	00116	6 0
IV.	4/21– 5/ 6/19	6	*00138*	*2 0*	5	*00117*	*4 0*	5	*00086*	*5 0*														6	*00545*	*7 4*			7	*00250*	*3 8*
V.	5/12– 5/27/19	6	00185	1 5	7	00137	3 0	4	00071	3 0										5	00117	5 4	4	00545	4 9						
VI.	6/11– 6/18/19	8	00172	1 7	8	00131	1 0	6	00101	1 7																					
VII.	10/1 –10/14/19	13	00177	1 5	11	00134	2 0	11	00097	3 7	4	0194	0 6	5	00852	2 0	4	0152	0 6												
VIII.	10/17–10/27/19	6	00168	1 5	6	00134	3 4	6	00094	4 0	4	0182	3 4	5	00774	2 5	4	0158	2 0												
IX.	11/1 –11/15/19	9	00181	1 3	9	00135	2 2	8	00096	5 0	6	0162	2 0	7	00739	1 3	8	0144	1 8												
X.	11/22–12/ 2/19	4	00179	1 4	5	00128	2 4	5	00061	5 7	3	0184	3 0	4	00772	2 8	4	0139	5 5												
XI.	12/3/19–1/6/20	7	00172	2 4	8	00127	2 8	8	00091	9 0	5	0187	5 8	7	00746	2 0	7	0164	5 0												
XII.	1/6 – 1/26/20	5	00161	4 5	5	00129	3 2	4	00077	6 5	5	0166	2 4	4	00737	3 2	4	0140	2 6												
XIII.	2/3 – 2/28/20	8	00179	1 9	8	00126	2 3	7	00063	6 3				4	00847	2 2	5	0159	3 9	8	00105	5 1	3	00666	1 6	7	000565 (Range 000507 to 000709)	5 3	5	00197	7 2

treated animals among those that were transferred from the country quarters have been discarded since the great discrepancy between these measurements and those in the earlier as well as in the later groups illustrates most strikingly the effect of variations in environment, season, etc. In this instance the effect of moving and of the changed environment was sufficient to put the animals in the abnormal class, although at autopsy no sign of disease could be discovered. We then began securing animals from a dealer in the country in small groups so that the measurements of all treated animals could be compared with the measurements of normal animals of the same group.

No conclusions have been drawn from findings in any series unless the difference between the electrical conductivity of the organ in the

TABLE 6

Range of electrical conductivity of various normal rabbit tissues arranged in the order of magnitude of their conductivity values

Spinal fluid	0.0164–0.0194
Bile	0.0139–0.0164
Blood	0.00739–0.00852
Voluntary muscle	0.00580–0.00745
Cerebrum	0.00161–0.00198
Cerebellum	0.00126–0.00151
*Heart	0.00105–0.00117
Liver	0.00061–0.00101
Lung	0.00051–0.00071

* On account of the wide range of the individual measurements of the heart muscle and the fact that in our earliest series its conductivity appeared to be higher, its place in this table may be questioned.

treated animal and the average measurement of the normal organ in the same group has been greater than the established average deviation of the normal measurements in that group.

It will be noted that throughout these researches, there has been no exception to what appears to be the normal relationship between the cerebrum and the cerebellum in the adult animal; i.e., in every adult animal the conductivity of the cerebrum has been greater than that of the cerebellum. This constant relationship was observed also by Doctor Obear in his preliminary studies. In order to discover whether or not this relation is a characteristic of adult life only, series of fetuses and of young rabbits were measured, and the significant observation was made that in fetuses and immediately after birth *the conductivity of the cerebellum was higher than that of the cerebrum.* In

TABLE 7

Relation between the electrical conductivity of the cerebrum and of the cerebellum in fetuses and in young rabbits

	CEREBRUM	CEREBELLUM	LIVER
Mother I	*00163*	*00125*	*00094*
Fetus 1	00087	00125	00049
2	00112	00137	00046
3	00103	00113	
4	00110	00119	00052
5	00070	00136	00048
Mother II	*00185*	*00138*	*00090*
Fetus 1	00097	00099	00033
2	00107		00033
3	00097	00099	
4	00096	00116	00057
Mother III (nearly at term)	*00143*	*00114*	*00099*
Fetus 1	00109	00115	00048
2	00086	00125	00059
3	00104	00119	00037
4	00076	00116	00058
5	00096	00108	00039
6	00092	00094	00045
Approximately 1–2 hours old			
1	00103	00120	00081
2	00096	00131	00088
24 hours old			
1	00122	00114	00072
2	00130	00116	00102
32 hours old			
1	00135	00118	00046
2	00107	00128	00059
4 days old			
1	00111	00140	00092
2	00132	00114	00072
3	00127	00094	00076
6 days old			
1	00145	00140	00078
2	00183		00075

TABLE 7—*Concluded*

	CEREBRUM	CEREBELLUM	LIVER
7 days old			
1	00165	00149	00068
2	00156	00162	00066
10 days old			
1	00159	00157	00102
2	00176	00132	00061
1 month, 20 days			
1	00171	00122	00084
2	00172	00120	00062
2-3 months			
1	00188	00158	00118
2	00195	00147	00099

most of the fetuses measured the conductivity of the cerebellum was as high as in the average adult, while the conductivity of the cerebrum in the fetus and in the newborn rabbits was far below normal. The rise of the conductivity of the cerebrum to the normal level apparently coincides with the emerging of the young rabbit from the nest and the inauguration of its conscious life as an independent individual (table 7, chart 1). As will be noted in the chart and table, the conductivity of the liver of the fetus is far below that of the normal adult, but apparently rises to the normal level at birth. It should be noted that on account of the very small size of the fetal cerebellum, it was necessary to use a special electrode and minute glass tubes, so that the possibility of error in the measurements of the cerebellum was greater than in the case of the cerebrum.

An interesting corollary to these observations in rabbits may be noted here. Permission was granted for securing sections of the brains of two patients who died in the hospital on the same day. One died from carcinoma of the stomach and had been conscious until death; the other had been unconscious for days before his death, which was caused by a brain tumor.

As is shown by table 8, in the patient conscious until death, the conductivities of the cerebrum and the cerebellum while low, undoubtedly as the result of the exhaustion of prolonged disease, nevertheless preserved the relationship observed in all our adult animals; viz., the

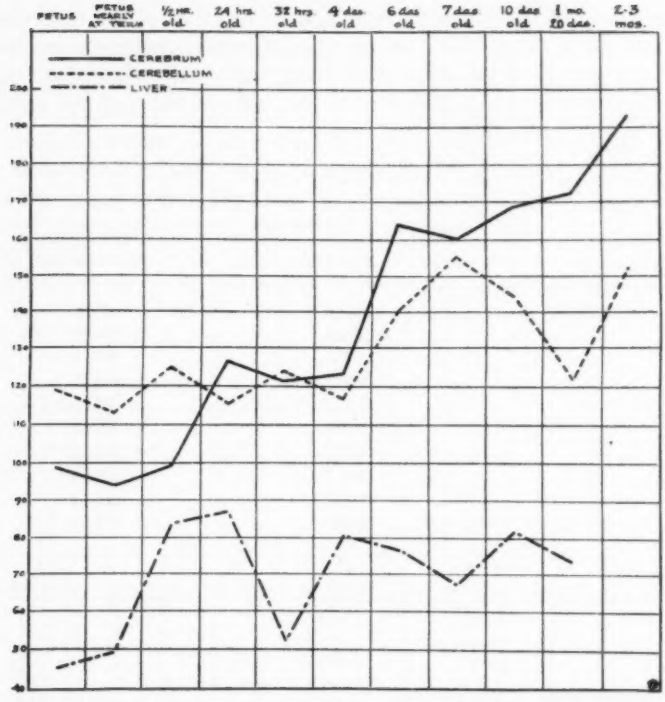

Chart 1. Progressive changes in the electrical conductivity of the brain and the liver of rabbits from just before birth to the age of 3 months.

TABLE 8

Comparison between the relative conductivities of the cerebrum and of the cerebellum of two patients—one conscious until death and one unconscious for days before death

	CEREBRUM	CEREBELLUM
I. Patient conscious until death		
Section I	00143	00116
Section II	00120	00116
Section III	00136	00107
II. Patient unconscious for days before death		
Section I	00139	00181
Section II	00135	00157
Section III	00134	00175

conductivity of the cerebrum was higher than that of the cerebellum. In the patient who had been unconscious, this relationship was reversed, the conductivity of the cerebellum being higher than that of the cerebrum. It will be noted also that in this case the value of the conductivity of the cerebellum is very high.

TABLE 9

Relation between the electrical conductivity of the gray matter and of the white matter of the cerebrum

SECTION	GRAY MATTER	WHITE MATTER
1	00213	00117
2	00218	00106
3	00209	00115
4	00227	00147
5	00215	00133
6	00271	00164
7	00202	00139
8	00232	00115
9	00260	00150
10	00240	00143

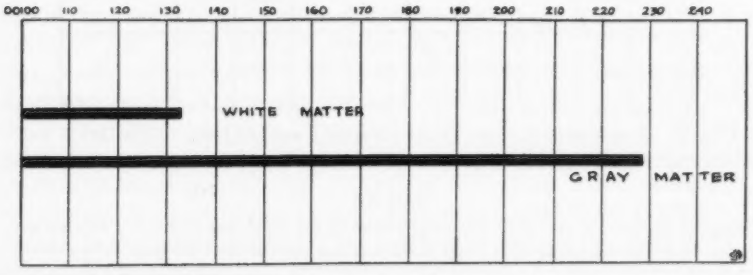

Chart 2. Comparison of the electrical conductivity of the gray and of the white matter of the cerebrum. (Actual measurements.)

A number of measurements has been made to determine whether or not there is a regional variation in the conductivity of the cerebrum. While these preliminary studies are suggestive, the only result which seems sufficiently established to be noted here is the relation between the conductivities of the gray and of the white matter. As shown by table 9 and chart 2, in every measurement thus far made, the conductivity of the gray matter has been markedly higher than that of the white matter.

EFFECTS OF EXHAUSTION DUE TO VARYING CAUSES UPON THE ELECTRICAL CONDUCTIVITY OF THE BRAIN AND THE LIVER. In selecting the types of exhaustion to be included in this research, we were guided by previous researches in order that we might discover whether or not any measurable relation could be established between the histological changes observed in exhaustion, in particular those in the brain, and the electrical conductivity. That this correlation might be fairly made, we have been constantly guided by the data of the previous researches in the treatment of the animals, dosage, protraction of stimulation, etc.

I. Insomnia. Thirteen Belgian hares of weights varying from 1.414 to 2.588 kgm. were kept awake continuously for 96 hours. During

TABLE 10

Effect of prolonged insomnia—96 hours—upon the weight and temperature of rabbits

RABBIT	INITIAL WEIGHT	FINAL WEIGHT	INITIAL	FINAL
	gm.	*gm.*	*temp.*	*temp.*
I	2.588	2.595	39.6	40.2
II	2.206	1.605	39.0	38.8
III	2.283	2.400	39.6	40.0
IV	2.243	2.254	39.0	39.0
V	2.158	2.215	39.6	39.6
VI	2.343	2.421	39.6	39.6
VII	2.223	2.321	39.6	39.2
VIII	1.730	1.715	39.0	40.0
IX	1.828	2.003	39.2	39.0
X	1.716	1.778	39.0	39.0
XI	1.793	1.630	39.2	39.0
XII	1.414	1.473	38.8	41.0
XIII	2.348	2.328	39.8	39.6

this time they were confined in a large airy room and were given abundant food and water. The animals were kept awake by constant but gentle prodding; they were not hurt in any way, nor at any time did they manifest any discomfort beyond their attempts to settle into corners where they might be left alone. Most of the animals ate and drank freely throughout the insomnia period. Table 10 gives the weight and temperature of each at the beginning and at the end of the period of insomnia.

At the end of the insomnia period four of the rabbits were killed at once and conductivity measurements made. Four were put into a darkened room and left undisturbed for 6 hours, when they in turn

TABLE 11

Effect of insomnia and of insomnia followed by periods of rest on the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUP	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
12/14–12/21/18	*Normal II*	*6*	*00189*	*1.4*		*6*	*00164*	*0.8*		*6*	*00072*	*5.9*	
a	Insomnia	4	00184	1.2	−2.6	4	00154	4.8	−6.09	5	00075	5.8	+4.1
b	Insomnia + 6 hours rest	5	00186	1.5	−1.5	5	00140	3.1	−14.6	5	00077	3.6	+6.9
c	Insomnia + 1–2 weeks rest	4	00203	4.0	+7.4	4	00155	3.0	−5.4	4	00089	3 9	+23.6

were killed and conductivity measurements made. The remaining four were kept undisturbed for from 7 to 14 days.

The average conductivities of the cerebrum, cerebellum and liver in each of these groups are shown in table 11 and chart 3.

II. Fright and exertion. Each of six rabbits was frightened until exhausted by a dog which kept them in a state of intense nervous excitement by barking and thwarted attacks until they were prostrated

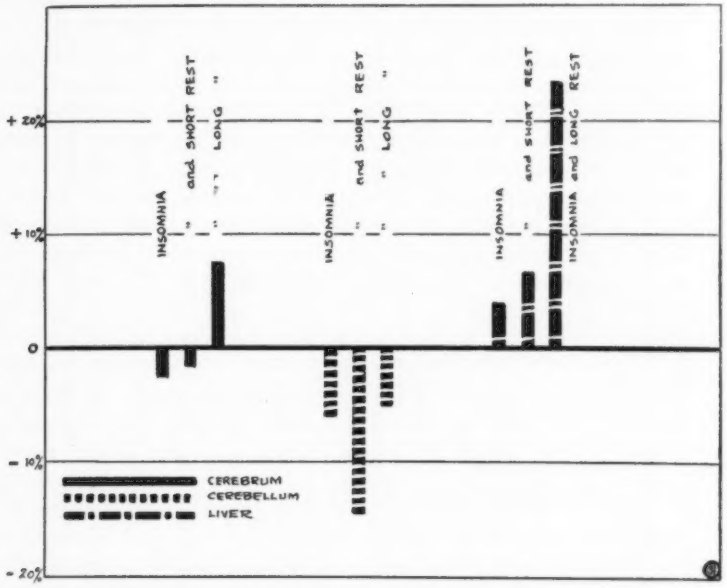

Chart 3. Comparison of the immediate effect of prolonged insomnia on the electrical conductivity of the brain and of the liver with the effect of short and of prolonged periods of rest. (Percentile variations from normal.)

by the resultant exhaustion. The effect upon the conductivity of the brain and the liver is shown in table 12 (a).

III. Adrenalin—repeated doses. Repeated doses—2 to 3—of 1-1000 adrenalin (P. D. & Co.) were given to each of 8 rabbits at intervals of from 10 to 20 minutes according to the degree of reaction. The average dose—intravenous—was 0.4 cc. per kgm.; to two rabbits twice this dose was given intramuscularly. Typical changes in pulse and respiration were produced in each animal with ultimate prostration. The conductivity changes are shown in table 12 (e).

TABLE 12

Effects of exhaustion from various causes upon the electrical conductivity of the liver

DATE OF EXPERIMENT	STIMULUS		NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
						per cent				*per cent*				*per cent*
12/14–12/21/18	*Normal II*		*6*	*00189*	*1.4*		*6*	*00164*	*0.8*		*6*	*00072*	*5.9*	
12/ 5–12/14/18	Fright	(a)	6	00180	3	− 4.7	6	00152	1.3	− 7.3	6	00118	8	+63.8
11/26–12/10/18	Insomnia	(b)	4	00184	1.2	− 2.6	4	00154	4.8	−6.09	4	00075	5.8	+ 4.1
1/ 3– 2/19/19	*Normal III*		*5*	*00192*	*1.6*		*5*	*00162*	*5.3*		*5*	*00074*	*2.2*	
1/ 8– 1/10/19	Surgical shock	(c)	5	00168	2.4	−12.5	5	00154	1.7	−4.9	6	00091	5.5	+22.9
12/ 8–12/18/19	Diphtheria toxin	(d)	5	00179	2.7	− 6.7	6	00156	3.1	−3.7	4	00069	3	− 6.7
1/ 3– 1/ 7/19	Adrenalin injection	(e)	8	00184	2	− 4.1	7	00154	1.4	−4.9	7	00094	3.4	+27.02
1/27– 2/17/19	Thyroid feeding	(f)	5	00175	2.5	− 8.8	5	00144	3.6	−11.1	5	00074	4.4	0
1/31– 2/ 8/19	Hydrochloric acid	(g)	9	00168	2.7	−12.5	9	00144	2.7	−11.1	9	00093	4	+25.6
1/30– 2/13/19	Ether—4 hours continuous	(h)	6	00177	1.6	− 7.8	6	00130	2.6	−19.7	6	00097	5.7	+31.08
2/13– 2/19/19	Nitrous oxid—4 hours continuous	(i)	6	00184	3.4	− 4.1	5	00139	2.3	−14.1	6	00094	7	+27.02
5/12– 5/27/19	*Normal V*		*6*	*00185*	*1.5*		*7*	*00137*	*3*		*4*	*00071*	*3*	
5/14– 5/26/19	Strychnin	(j)	5	00146	3.9	−21.08	4	00130	5.4	−5.1	5	00092	3.3	+29.5

IV. Surgical shock. Each of six rabbits was subjected, under ether, to severe trauma of the intestines and abdominal walls for periods of from 30 to 45 minutes. The resultant conductivity changes are shown in table 12 (e).

V. Prolonged ether and prolonged nitrous oxid anesthesia. Six rabbits were subjected to 4 hours' continuous ether anesthesia; and six to continuous nitrous oxid anesthesia of the same duration with resultant conductivity changes which are shown in table 12 (h) and (i).

VI. Thyroid feeding. Six rabbits in fine general condition and of approximately equal weight were each given 5 grains of thyroid extract daily for 3 weeks. All but one showed at first a loss of appetite, with a later increased appetite but a continued loss of weight. With the exception of the one referred to above, which seemed to thrive, the fur of all became rough and coarse in appearance and was shed abundantly; they became nervous and excitable; the eyes were staring in appearance; the skin felt hot to the touch although the clinical thermometer showed no change in temperature. In brief, the animals manifested the typical signs of thyroid intoxication. The probable reason for the exception of the one rabbit noted above appeared at autopsy, which showed a minute and pale thyroid gland as compared with enlarged vascular glands in each of the others.

The losses of weight in each animal were as follows: 18 per cent, 29 per cent, 15 per cent, 36.6 per cent, with a loss of but 4.5 per cent in the exceptional one. One animal died after 2 weeks, with a loss in weight of 33.8 per cent. At the termination of 3 weeks the animals were killed and conductivity measurements made (table 12 (f)). It should be noted that in this group the thyroid feeding was protracted until the stage of exhaustion had been reached. Earlier effects are described in a later section.

VII. Hydrochloric acid. In each of four rabbits 1 cc. of hydrochloric acid—10 per cent—was injected in the femoral vein. Each showed an immediate reaction registered in circulatory and respiratory changes, convulsive movements and prostration. The animals were killed in from four to twelve minutes after the injection and conductivity measurements made (table 12 (g)).

VIII. Strychnin. On account of the wide variation in the response of individual rabbits to strychnin, in this series in which it was desired to produce a massive effect the dosage varied from the just tetanic (0.155 mgm. per kgm. intravenous, Sollman) to the just fatal (0.36 mgm. per kgm. intravenous, Sollman), the dose being repeated if required to

produce sufficient reaction. Five animals were included in the series. In each a reaction varying from a general tremor to severe convulsions was produced.

While on account of the variation in dosage and clinical results this is not considered a satisfactory series, the contrast in the conductivity findings to those in a subsequent series in which the incipient effects were noted, is so marked that the series has been included. (Table 12, (j)).

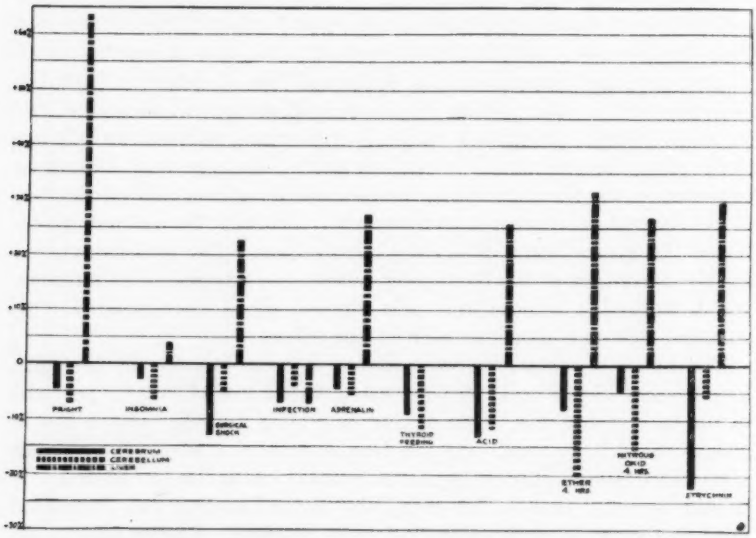

Chart 4. Effects of exhaustion due to varying causes upon the electrical conductivity of the brain and liver. (Percentile variations from the normal.) Note that in only one instance—infection—is the conductivity of the liver decreased: in thyroid feeding the average conductivity of the liver was unchanged.

SUMMARY. A study of table 12 and chart 4 shows that in each type of exhaustion there included the conductivity of the cerebrum and of the cerebellum was diminished and the conductivity of the liver was increased except in exhaustion due to thyroid feeding in which case the average conductivity of the liver was unchanged from the normal. Chart 5 shows that with the exception of exhaustion produced by adrenalin injection and by prolonged nitrous oxid anesthesia, in every instance the average conductivity of the cerebrum in exhaustion fell

beyond the lowest individual normal measurement included in the normal group with which comparison is made. If one allows for the average deviation of the cerebellum and of the liver in both the exhausted and the normal animal, in certain instances the apparent change in exhaustion falls within those limits, nevertheless the marked downward tendency in the cerebrum and the cerebellum, and the upward tendency in the liver are obvious, as is shown also in chart 5 in which are charted the actual average deviations from the normal for all measurements in which normal group III was the basis for comparison.

INCIPIENT EFFECTS OF EXHAUSTION-PRODUCING AGENTS ON THE ELECTRICAL CONDUCTIVITY OF THE BRAIN AND THE LIVER. Previous researches and clinical observations had indicated the presence of an incipient stage of shock marked by hyperchromatism of the brain cells

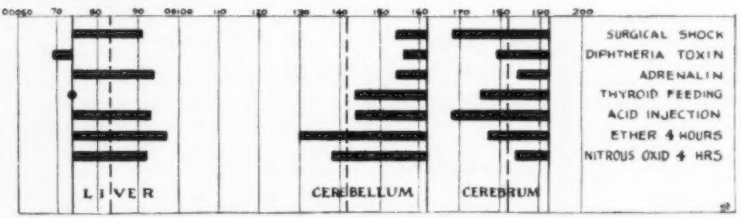

Chart 5. Changes in the electrical conductivity of the brain and of the liver in exhaustion from various causes. (Actual deviations from the normal.) Note the vertical dotted lines which indicate the average deviation of each tissue from the normal average indicated by the solid vertical lines.

as compared with hypochromatism after shock had become established. To determine whether or not a corresponding early increase in the electric conductivity of the brain precedes the ultimate decreased conductivity after the state of exhaustion or shock is established, a number of studies of the incipient effects of some of the shock-producing agents noted above was made.

I. Incipient effects of intense trauma. Under light ether anesthesia two groups of rabbits were subjected to extreme shock-producing manipulations for periods of 1 and of 5 minutes respectively. They were killed immediately and electric conductivity measurements made (table 13 (a), chart 6).

Incipient effects of strychnin. Each of five rabbits was given a fatal dose (0.36 mgm. per kgm.) intravenously and killed 1 minute after the injection (table 13 (d) chart 6).

TABLE 13

Incipient effects of various exhaustion producing agents upon the electrical conductivity of the brain and the liver

NUMBER AND DATE OF NORMAL GROUP FOR COMPARISON	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
6/11–6/18/19	*Normal VI*	8	*00172*	*1.7*		8	*00131*	*1*		6	*00101*	*1.7*	
6/ 9/19	(a) Surgical shock—1 min.	5	00189	1.0	+ 9.8	6	00138	1.3	+5.3	4	00108	1.9	+ 6.9
6/ 6/19	5 min.	3	00175	0.8	+ 1.7	3	00142	1.9	+8.3	3	00116	6.5	+14.8
6/10/19	(b) Ether—stage of excitement	3	00195	0.6	+13.3	3	00126	2.3	−3.8	2	00119	4.2	+17.8
6/11/19	(c) Nitrous oxid—stage of excitement	3	00174	1.6	+ 1.1	3	00139	6.3	+6.1	3	00099	6.6	− 1.9
6/12–6/16/19	(d) Strychnin	4	00193	3.0	+12.2	4	00143	2.1	+9.1	3	00092	7.3	− 8.9
6/13–6/16/19	(e) Adrenalin	4	00181	3.4	+ 5.2	4	00129	2.7	−1.5	4	00095	4.6	− 5.9

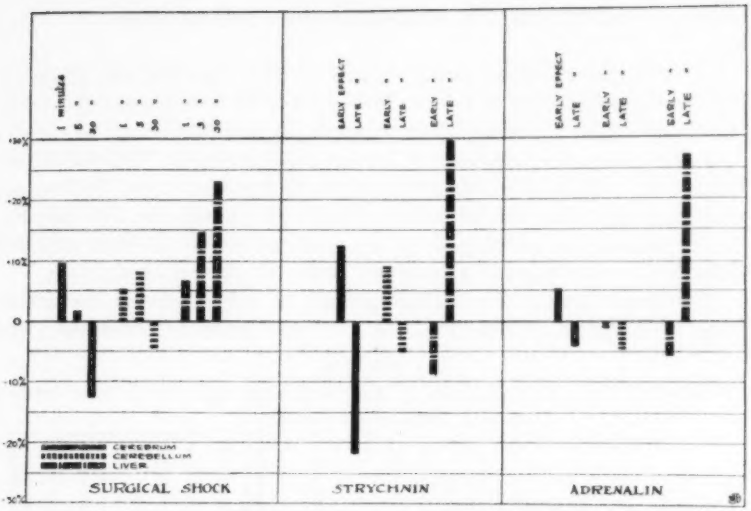

Chart 6. Comparison of the incipient and late effects of stimulation by various agents on the electrical conductivity of the brain and of the liver. (Percentile variations from the normal.)

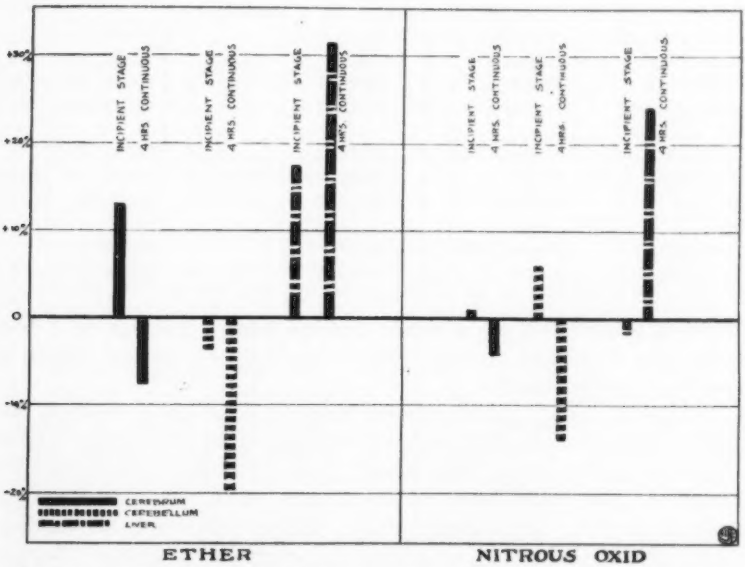

Chart 7. Incipient and late effects of ether and of nitrous oxid-oxygen anesthesia on the electrical conductivity of the brain and the liver. (Percentile variations from the normal.)

Incipient effects of adrenalin. Each of three rabbits was given an intravenous injection of 0.4 cc. per kg. of 1-1000 adrenalin (P. D. & Co.) and killed 1 minute later (table 13 (e) chart 6).

Incipient effects of ether and of nitrous oxid anesthesia. To one of two groups of rabbits ether was administered for from 2 to 5 minutes; nitrous oxid being administered to the other group for like periods. Each animal was killed just at the termination of the first stage of anesthesia—the stage of excitement (table 13 (b and c) chart 7).

Diphtheria toxin. To various groups of rabbits twice the lethal dose of diphtheria toxin (P. D. & Co.) was given intravenously, the animals being killed at intervals varying from 5 minutes to 1 hour after the dose was received—the progressive effects are shown in table 14 and in chart 8.

TABLE 14

Progressive effects of the injection of diphtheria toxin upon the electrical conductivity of the brain

DATE OF EXPERIMENT	PERIOD AFTER INJECTION OF TOXIN	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*
5/12–5/27/19	*Normal V*	*6*	*00185*	*1.5*		*7*	*00137*	*3*	
6/2–6/4/19	15 min.	2	00226	1.9	+22.0	3	00137	0.9	0
6/2–6/4/19	30 min.	3	00194	1.3	+ 4.8	3	00128	1.8	− 6.5
5/27–5/29/19	1 hour	3	00180	1.7	− 2.7	3	00109	0.5	−20.4
12/3/19–1/6/20	*Normal XI*	*7*	*00172*	*2.4*		*8*	*00127*	*2.8*	
12/15/19–1/2/20	15 min.	3	00189	1.2	+ 9.8	3	00154	1.7	+21.2
1/7/20	30 min.	2	00174	3.8	+ 4	2	00131	0	+ 3.1
1/3–2/19/19	*Normal III*	*5*	*00192*	*1.6*		*5*	*00162*	*5.3*	
2/8–2/18/19	4 hours	5	00179	2.7	− 6.7	6	00156	3.1	− 3.8

SUMMARY. With every exhaustion-producing agent studied, the initial effect was an increased conductivity of the cerebrum followed by a decrease to below the normal when the stage of exhaustion was reached. The early effect of stimulation upon the cerebellum appeared to vary, but a study of the clinical behavior of the animals, especially of the initiation of the respiratory and circulatory changes, together with an examination of the individual measurements, would

seem to indicate that a like unvarying rule exists in the case of the cerebellum, but that the protraction of the incipient stage is shorter than in the case of the cerebrum. Many additional experiments are required to establish this point.

A study of the individual measurements of the liver would appear to indicate an immediate tendency to decrease followed by an increase to above the normal.

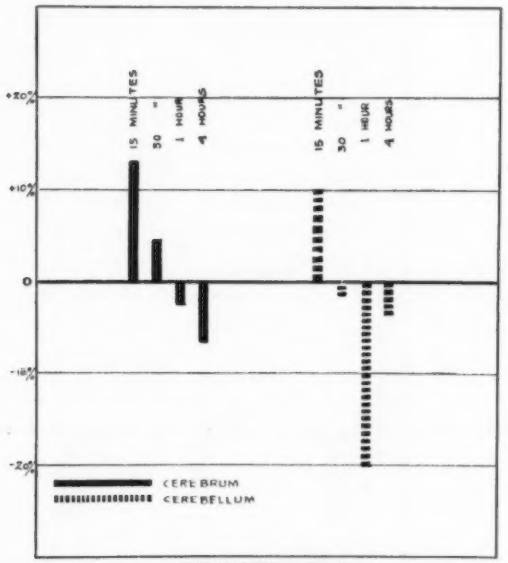

Chart 8. The incipient and later effects of the injection of diphtheria toxin on the electrical conductivity of the brain. (Percentile variations from the normal.)

THE EFFECTS OF DIPHTHERIA TOXIN IN THE PRESENCE OF MORPHIN ON THE ELECTRICAL CONDUCTIVITY OF THE BRAIN AND LIVER. The control of infection by morphin, so strikingly illustrated by the Alonzo Clark treatment of peritonitis, and the comparison of the histologic effects of diphtheria toxin alone and in the presence of morphin, led us to perform a series of experiments to determine whether or not the protective effect of morphin would be manifested by any diminution of the conductivity changes produced by diphtheria toxin alone.

TABLE 15

Comparison of the effects of diphtheria toxin alone and of diphtheria toxin in the presence of morphin on the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUP	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
12/14–12/21/18	*Normal III*	*6*	*00192*	*1.6*		*6*	*00162*	*5.3*		*3*	*00074*	*2.2*	
2/8 –12/18/19	Diphtheria toxin	5	00179	2.7	−6.7	6	00156	3.1	−3.7	4	00069	3.0	− 6.7
2/6 – 2/13/19	Morphin	6	00185	4.6	−3.6	6	00148	4.1	−8.6	6	00065	6.9	−12.2
2/6 – 2/18/19	Diphtheria toxin and morphin	6	00187	2.6	−2.6	6	00164	3.2	+1.2	6	00075	4.2	+ 1.4

Coincidently with the series of experiments described above in which the rabbits were killed 4 hours after the intravenous injection of twice the lethal dose of diphtheria toxin, to each of another group 5 grains of morphin were given hypodermically in two doses, 1 hour apart; twice the lethal dose of diphtheria toxin being given intravenously

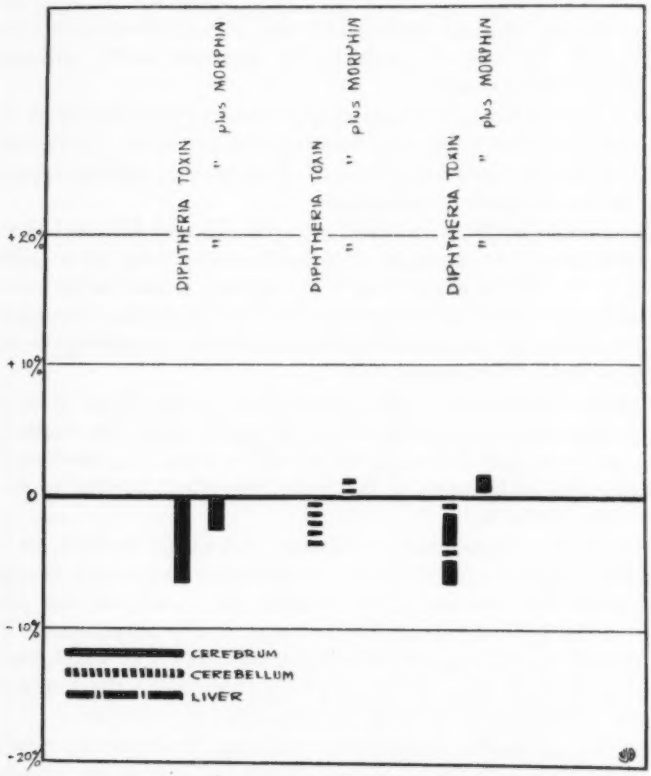

Chart 9. Comparative effects of the injection of diphtheria toxin alone and in the presence of morphin on the electrical conductivity of the brain and the liver. (Percentile variations from the normal.)

15 minutes after the second dose of morphin was received. The animals were killed 4 hours after receiving the diphtheria toxin.

To each of a third group morphin alone was administered as above, and the animals killed 4 hours after the second dose. The conductivity measurements are given in table 15, and illustrated in chart 9.

EFFECTS OF THYROID FEEDING, OF IODIN AND OF ADRENALIN IN THE PRESENCE OF EACH, UPON THE ELECTRIC CONDUCTIVITY OF THE BRAIN AND THE LIVER. In a preceding section we have shown that the late effects of thyroid feeding are identical with the late effects of other exhaustion-producing agents.

Three later series of experiments were performed to discover the earlier effects of thyroid feeding and what if any, effect upon the conductivity of the brain is produced by adrenalin in the presence of thyroidism, or of iodism.

The results of these series are shown in tables 16 and 17 and in charts 10 and 11, in which have been included also for ready comparison the effects of thyroid feeding to the point of exhaustion, and the immediate effect of the injection of adrenalin.

The animals included in table 16, series II and III, and chart 10 had been given 2 to 3 grains of thyroid extract daily for a period of 4 weeks. It will be noted that while thyroid extract alone increases the conductivity of the cerebrum and of the cerebellum, the injection of adrenalin in the thyroid-fed animals produced a tendency to return toward or below the normal.

In table 17 and chart 11 the same contrast in the effects of iodoform and of iodoform plus the injection of adrenalin upon the conductivity of the cerebrum and of the cerebellum will be noted; i.e., iodoform alone increases the conductivity of the brain, this effect tending to be neutralized by adrenalin.

Each of the animals in the iodoform series had received an intraperitoneal injection of 75 grams of iodoform introduced through a small abdominal opening. The incision was closed and the animals killed on the following day. Each showed febrile phenomena. There was an early rise of temperature of from 0.4 to 1.3°C. which was persistent in all but three of the cases; in those three the temperature dropped to from 0.1 to 1.0°C. below the initial temperature.

That the increased conductivity produced by iodoform cannot be due to the permeation of the tissues by the iodine is shown in series II, table 17, in which, while the conductivity of the cerebrum and of the cerebellum is markedly increased, the conductivity of the liver is practically unchanged.

An essential corollary to these experiments would be the measurement of the conductivity of the brain in thyroidectomized animals after the injection of iodoform.

TABLE 16

Early and late effects of thyroid feeding and thyroid feeding plus adrenalin upon the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
Series I													
1/3 – 2/19/19	*Normal III*	5	*.0192*	*1.6*		5	*.0162*	*5.3*		5	*.0074*	*2.2*	
1/27– 2/17/19	Thyroid feeding, late effects	5	.0175	2.5	– 8.8	5	.0144	3.6	–11.1	5	.0074	4.4	0
Series II													
12/3/19–1/6/20	*Normal XI*	7	*.0172*	*2.4*		8	*.0127*	*2.8*		8	*.0091*	*9*	
12/3 –12/ 9/19	Thyroid feeding	3	.0189	0.9	+ 9.8	3	.0147	0.9	+15.7	4	.0080	2.5	–12.08
12/3 –12/ 5/19	Thyroid feeding plus adrenalin	2	.0188	0.3	+ 9.3	3	.0127	1.1	0	2	.0070	0	–23.07
Series III													
2/3– 2/28/20	*Normal XIII*	8	*.0179*	*1.9*		8	*.0126*	*2.3*		7	*.0063*	*6.3*	
2/17– 2/18/20	Thyroid feeding	2	.0199	2.6	+11.1	1	.0137		+ 8.7	2	.0061	4.1	– 3.1
2/17– 2/18/20	Thyroid feeding plus adrenalin	3	.0163	2.1	– 8.9	3	.0128	2.3	+ 1.6	3	.0050	11.3	–20.6
Early effects of adrenalin alone													
6/11– 6/18/19	*Normal VI*	8	*.0172*	*1.7*		8	*.0131*	*1*		6	*.0101*	*1.7*	
6/13– 6/16/19	Adrenalin	4	.0181	3.4	–33.4	4	.0129	2.7	– 1.5	4	.0065	4.6	– 5.9

TABLE 17

Effect of iodoform and of iodoform plus adrenalin on the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL
					per cent				*per cent*				*per cent*
Series I.													
6/11–6/18/19	*Normal VI*	*8*	*00172*	*1.7*		*8*	*00131*	*1*		*6*	*00101*	*1.7*	
6/17–6/18/19	Iodoform	3	00193	0.5	+12.2	4	00134	1.1	+ 2.2	4	00131	7.3	+29.7
Series II.													
2/ 3–2/28/20	*Normal III*	*8*	*00179*	*1.9*		*8*	*00126*	*2.3*		*7*	*00063*	*6.3*	
2/ 6/20	Iodoform	2	00194	4	+ 8.3	2	00140	4	+11.1	2	00064	6.7	+ 1.5
2/ 5/20	Iodoform plus adrenalin	2	00176	2.2	− 1.6	2	00124	0	− 1.5	2	00083	1.2	+31.7
Early effects of adrenalin alone													
6/11-6/18/19	*Normal VI*	*8*	*00172*	*1.7*		*8*	*00131*	*1*		*6*	*00101*	*1.7*	
6/13–6/16/19	Adrenalin	4	00181	3.4	+ 5.2	4	00129	2.7	− 1.5	4	00095	4.6	− 5.9

OPPOSITE EFFECTS OF ACID AND OF ALKALI INJECTION UPON THE ELECTRICAL CONDUCTIVITY OF THE BRAIN AND THE LIVER. In each of 6 rabbits 10 cc. of a saturated solution of sodium bicarbonate were slowly injected through the marginal ear vein. Each animal was killed 2 hours after the injection and sections taken for conductivity measurements.

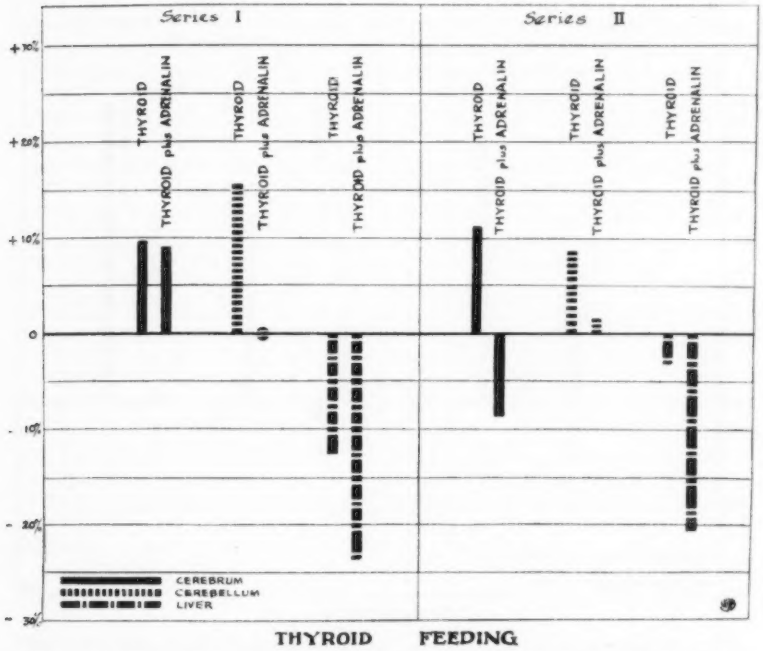

Chart 10. Changes in the electrical conductivity of the brain and of the liver produced by thyroid feeding and by thyroid feeding plus the injection of adrenalin. (Percentile variations from the normal.)

As will be seen in table 18 and chart 12, the injection of sodium bicarbonate increased the conductivity of the brain and decreased the conductivity of the liver, while the injection of hydrochloric acid, as described in a preceding section of this report, decreased the conductivity of the brain and increased the conductivity of the liver.

MISCELLANEOUS GROUP—SHOWING THE EFFECTS OF VARIOUS AGENTS UPON THE ELECTRIC CONDUCTIVITY OF THE BRAIN. In this section are

included a number of preliminary studies. No comment is made, as they should be extended before any conclusions can be drawn. The indications are sufficiently shown in tables 19 and 20.[1]

I. Magnesium sulphate—calcium chloride. To each of four rabbits 6 cc. per kgm. of a 25 per cent solution of magnesium sulphate was given intramuscularly. The animals were killed one-half hour after

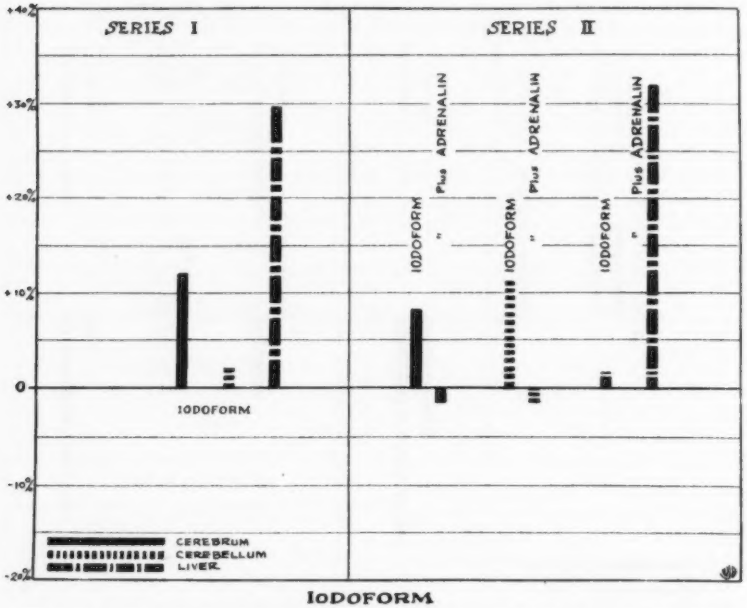

Chart 11. Changes in the electrical conductivity of the brain and of the liver produced by iodoform and by iodoform plus injection of adrenalin. (Percentile variations from the normal.)

the anesthesia was complete and sections taken for conductivity measurements.

To each of another group of four rabbits a like dose of magnesium sulphate was given, followed, one-half hour after anesthesia was complete, by the intravenous injection of 8 cc. (per kgm.) of a 3 per cent

[1] Sollman's *Laboratory Guide in Pharmacology* was used as a guide in determining the dosage in each group of experiments included in this section.

TABLE 18

Comparison of the effect of an acid with the effects of an alkali upon the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL
					per cent				*per cent*				*per cent*
6/11–6/18/19	*Normal VI*	8	*00172*	*1.7*		8	*00131*	*1.0*		6	*00101*	*1.7*	
6/19–6/20/19	Sodium bicarbonate	6	00178	2.2	+ 3.4	6	00139	3.1	+ 6.1	6	00087	6.6	−13.8
1/ 3–2/19/19	*Normal III*	5	*00192*	*1.6*		5	*00162*	*5.3*		5	*00074*	*2.2*	
1/31–2/ 8/19	Hydrochloric acid	9	00168	2.7	−12.5	9	00144	2.7	−11.1	9	00093	4.0	+ 8.1

TABLE 19

Comparison of the effects of magnesium sulphate and of magnesium sulphate plus calcium chloride

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
2/ 3–2/28/20	*Normal XIII*	*8*	*00179*	*1.9*		*8*	*00126*	*2.3*		7	*00063*	*6.3*	
2/19–2/27/20	$MgSO_4$	4	00164	1.2	−8.3	3	00130	2.2	+3.1	4	00064	1.7	+ 1.5
	$MgSO_4 + CaCl_2$	4	00171		−4.4	4	00116	2.1	−7.9	3	00054	5.8	−14.2

solution of calcium chloride. In each case the animal became completely conscious almost immediately after the calcium chloride injection. It was killed at once and conductivity measurements made.

The contrasting results in the two series are shown in table 19.

II. Calomel. To each of three rabbits 2 cc. (per kgm.) of a 0.5 per cent solution of calomel in sodium thiosulphate was given hypodermically. The animals were killed 24 to 30 hours later. Each showed marked diuresis and diarrhea, with some salivation (table 20 a).

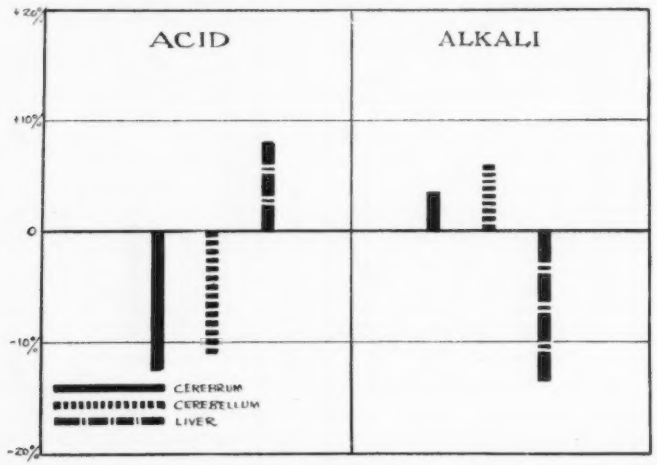

Chart 12. Opposite effects of the injection of an acid and of an alkali on the electrical conductivity of the brain and of the liver. (Percentile variations from the normal.)

III. Caffein. (a) To each of 5 rabbits 4 cc. (per kgm.) of a 1 per cent solution of caffein was given intravenously. Each showed extreme toxic effects and died within 10 to 15 minutes.

(b) To each of four rabbits 1 to 2 cc. (per kgm.) of the 1 per cent caffein solution was given hypodermically in two successive doses about 10 minutes apart, the interval determined by the clinical effects. These showed less marked but positive clinical effects terminating in milder convulsions. The animals were killed when the convulsions appeared.

(c) To each of five rabbits two or three doses as in the last preceding series were given and the animals kept until the following day, when a

TABLE 20

Effects of various agents upon the electrical conductivity of the brain

DATE OF EXPERIMENT, GROUP	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL
				per cent					*per cent*
11/ 1-11/15/19	*Normal IX*	*9*	*00181*	*1.3*		*9*	*00135*	*2.2*	
10/28-10/29/19	(a) *Calomel*	3	00166	1.0	− 8.2	3	00136	0.3	+ 0.7
10/29-11/21/19	(b) *Caffein*								
	Single massive dose	4	00163	1.5	− 9.9	4	00.42	1.6	+ 5.1
	Repeated doses								
	1 hour	4	00177	2.2	− 2.2	4	00141	1.5	+ 4.4
	Repeated 2 days	5	00158	1.6	−12.7	5	00.42	3.2	+ 5.1
11/22-12/ 2/19	*Normal X*	4	00179	1.4		5	00128	2.4	
11/19-12/ 6/19	(c) *Sodium bromid*								
	1 hour	3	00184	1.8	+ 2.7	3	00128	4.1	0
	2 hours	3	00180	0.9	+ 0.5	2	00126	0.5	− 1.5
	15-16 hours	2	00180	0	+ 0.5	2	00145	1.1	+13.2
	24 hours	2	00174	4	− 2.7	2	00127	4.6	− 0.7
	48 hours	2	00170	1.4	− 5.02	2	00119	0.6	− 7.03
1/ 6- 1/26/20	*Normal XII*	*5*	*00161*	*4.5*		*5*	*00129*	*3.2*	
1/15- 1/17/20	(d) *Cocaine*								
	Intravenous	4	00167	0.7	+ 3.7	4	00133	1.4	+ 3.1
	Intramuscular	2	00155	2.8	− 3.7	2	00115	1.5	−10.8

final dose was given and the animal killed during the resultant convulsion.

The average effects of the caffein upon the electrical conductivity of the animals in each of these groups is shown in table 20 b.

IV. Sodium bromid. Twelve rabbits were divided into 5 groups and given sodium bromid (3 gm. per kgm.) through a stomach tube in single or repeated doses, as follows:

Group 1, 3 rabbits, 1 dose—killed after 1 hour.
Group 2, 3 rabbits 2 doses—1 hour apart—killed 1 hour after last dose.
Group 3, 2 rabbits, 1 dose—killed 15-16 hours later.
Group 4, 2 rabbits, 1 dose on each of two successive days—killed 1 hour after second dose.
Group 5, 2 rabbits, 1 dose on each of 4 successive days—killed 1 hour after last dose.

The average effects upon the electric conductivity of the brains of the animals in each group are shown in table 20 c.

V. Cocaine. (a) To each of 4 rabbits 1.2 cc. (per kgm.) of a 5 per cent solution of cocaine was injected intravenously. The injection was followed by a mild convulsion followed by complete relaxation. The animals were killed in from one-half to three-quarters of an hour after the dose was received.

(b) To each of two rabbits 2 cc. (per kgm.) of a 5 per cent solution of cocaine was injected intramuscularly, and the animals killed one hour later (table 20 d).

MEASUREMENTS OF THE ELECTRIC CONDUCTIVITY OF PATHOLOGICAL HUMAN TISSUES. In view of the finding of Loeb, Lillie, McClendon and other physical chemists that the permeability of the ovum is increased by fertilization, and the indication of their researches and our own that any alteration in function of the cells is attended by an alteration in their electric conductivity, one would infer that the electric conductivity of tissues in which the cells are in as active a state as in cancerous tissue would be higher than the electric conductivity of normal tissue. We therefore included in our research measurements of the electric conductivity of malignant and benign tumors and of various precancerous conditions.

In this study we have measured 219 sections from 159 clinical cases. These have included malignant and benign tumors of the breast and of the uterus, ulcer and carcinoma of the stomach, carcinoma of the rectum, malignant and benign tumors of the mouth, jaws, and neck, x-ray burns and various types of goiters—hyperplasia, fetal adenoma,

multiple adenoma, toxic adenoma, exophthalmic goiter, simple colloid goiter, thyroiditis. Whenever possible adjacent normal tissue has been measured for comparison. The pathological diagnosis and differentiation of different types of tissue in single specimens were made by the pathologist of the surgical section at Lakeside Hospital.

Among the goiters the highest conductivities were found among the adenomata. Variations in conductivity are well illustrated by the following measurements of a goiter, one portion of which was malignant. Three specimens from this gland gave the following measurements:

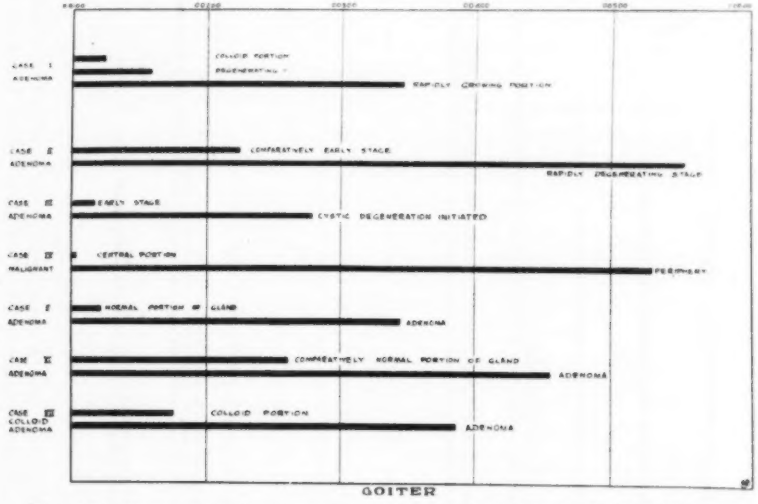

Chart 13. Comparison in seven cases of the electrical conductivity of the comparatively normal or inactive portions of a thyroid gland with the conductivity of the rapidly growing or degenerating portion of the same gland. (Actual measurements.)

colloid portion, 0.00124; early degeneration, 0.00159; rapidily growing portion, 0.00346.

The highest conductivities were found in the degenerating adenomata and the malignant thyroids; the conductivities of the hyperplastic thyroids were lower; and the conductivities of the colloid goiters were the lowest of any of the pathological tissues studied.

In all instances in which comparative measurements were made the conductivity of the malignant growth was higher than that of a normal portion of the same organ, as is illustrated by the following examples (charts 13 to 15):

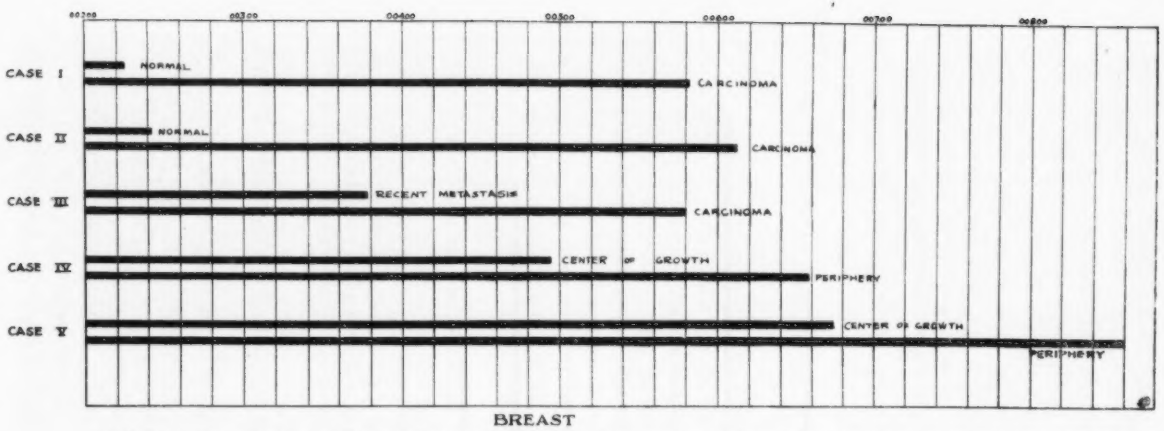

Chart 14. Comparison of the electrical conductivity of normal and of carcinomatous breast tissues and of different stages in the development of carcinoma of the breast. (Actual measurements.)

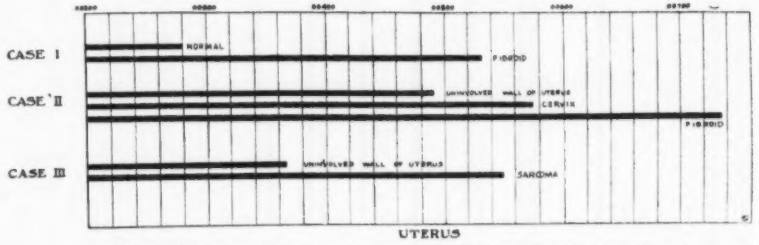

Chart 15. Comparison in three cases of the electrical conductivity of the comparatively normal portion of the uterus with a tumorous portion of the same uterus. (Actual measurements.)

Sarcoma of the uterus:
Comparatively normal portion 0.00367
Sarcomatous portion 0.00550
Mixed tumor of parotid:
Comparatively normal gland 0.00153
Malignant portion (2 sections) 0.00922, 0.01058
Carcinoma of the breast:
Comparatively normal portion 0.00225
Carcinomatous portion 0.00581
Carcinoma of the pylorus:
Normal mucosa 0.00126
Base of growth 0.00469

In the light of these comparisons the following measurements of the conductivities of x-ray burns and of the adjacent normal tissues are significant (chart 16).

Case I. Normal tissue, 0.00103; x-ray burn, 0.00717.
Case II. Normal tissue, 0.00151; x-ray burn, 0.00366.

The outer growing parts of cancers showed a high conductivity in contrast with the conductivity of the central non-growing parts.

Carcinoma of the thyroid:
Center of growth 0.00101
Periphery 0.00528
Carcinoma of the uterus:
Center of growth 0.00493
Periphery 0.00658

SUMMARY AND CONCLUSIONS

1. In an attempt to determine the specific electric conductivity of various normal animal tissues and whether or not variations in function are accompanied by measurable changes in their electric conductivity, 4764 sections from 455 rabbits and 219 sections of pathological human tissues have been measured.

2. The specific normal conductivity of the cerebrum, cerebellum and liver can be estimated within a narrow range; while the normal conductivity of other tissues can be estimated within a sufficiently narrow range to determine the order of their relative conductivities.

3. The spinal fluid has the highest conductivity of any of the tissues studied, the lung and the liver the lowest.

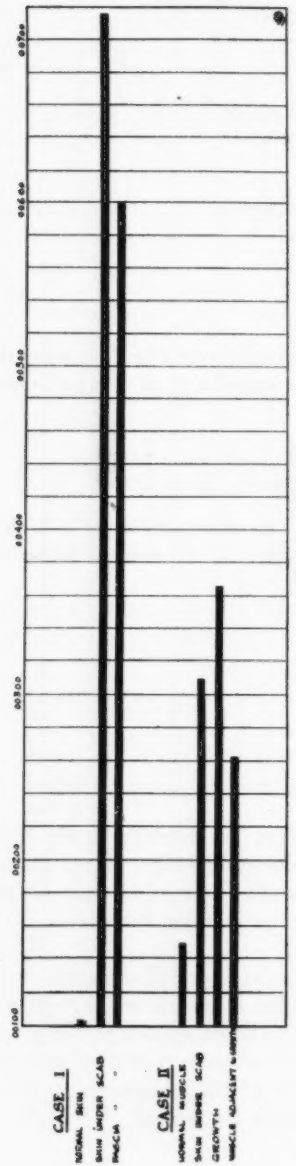

Chart 16. Comparison of the electrical conductivity of normal tissues with tissues involved in x-ray burns. (Actual measurements.)

4. The order of the conductivities of the following tissues was unchanged in all the animals studied with the exception noted in (6); viz., spinal fluid, bile, blood, voluntary muscle, cerebrum, cerebellum, liver, lung. In a limited number of observations the conductivity of the heart fell between that of the cerebellum and the liver, but on account of the wide range of the individual measurements, this cannot be considered as established.

5. The conductivity of normal tissues appears to vary according to the season and the general environment.

6. In every normal adult animal studied the conductivity of the cerebrum was higher than the conductivity of the cerebellum. In fetuses and in very young rabbits this relation was reversed—the conductivity of the cerebellum being higher than the conductivity of the cerebrum until about the time when the young rabbit left the nest and began voluntary activities, when the normal adult conductivity relation of the cerebrum and the cerebellum appeared to be established. A most significant corollary to this observation was found in the post-mortem examination of the brains of two patients, one of whom died after days of unconsciousness resulting from a brain tumor, while the other who died from carcinoma of the stomach, was conscious to the end. In the patient who had been unconscious, *the conductivity of the cerebellum was higher than that of the cerebrum.* In the other patient, as in all our normal animals, the conductivity of the cerebrum was higher than that of the cerebellum.

7. The conductivity of the gray matter of the brain is higher than that of the white matter.

8. Exhaustion from any cause—surgical shock, insomnia, emotion (fright), infection, etc.—is marked by a *diminished conductivity of the brain* and an increased conductivity of the liver.

9. The immediate effect of activation appears to be an increased conductivity of the brain, tending later to decrease as the stage of exhaustion approaches. As the charts indicate, this has been shown to be an immediate effect of physical injury; an early effect of the injection of diphtheria toxin; an immediate effect of the injection of adrenalin.

10. Thyroid feeding in large doses over a prolonged period produces the typical symptoms of hyperthyroidism with ultimate exhaustion accompanied by the changes in the conductivity of the brain typical of exhaustion from any other cause; i.e., the conductivity of the cerebrum and cerebellum is decreased.

11. Thyroid feeding in moderate doses until the symptoms of hyperthyroidism appear but not to the stage of exhaustion produces conductivity changes in the brain typical of the stage of stimulation produced by other agents; i.e., increased conductivity of the brain and decreased conductivity of the liver. These changes were diminished or reversed by the administration of adrenalin.

12. Iodoform increases the conductivity of the brain and the liver. These changes are reversed by adrenalin.

13. The injection of hydrochloric acid produced *diminished conductivity of the cerebellum and cerebrum and increased conductivity of the liver.* The injection of sodium bicarbonate produced *increased conductivity of the cerebellum and cerebrum and decreased conductivity of the liver.*

14. Rabbits were kept awake continuously for 96 hours. At the end of this period a number were killed and conductivity measurements made; others were allowed a brief period of rest of from four days to a week. At the end of the insomnia period the *conductivity of the brain was decreased* and the conductivity of the liver was increased; at the end of the short period of rest, the conductivity of the brain and of the liver was but little changed, if at all; at the end of the longer periods of rest, the brain was again approaching its normal conductivity.

15. A limited number of observations indicate that the changes produced by infection are minimised provided the infection is applied in the presence of morphin: that is, excessive infection alone decreases the conductivity of the brain and increases the conductivity of the liver; in this limited series of observations the conductivity of the brain remained practically unchanged when diphtheria toxin was administered in a morphinised animal, and the conductivity of the liver was but slightly altered.

16. A limited series of observations of the influence upon the electric conductivity of the brains of animals of various agents, which produce marked clinical effects, indicate that the progress of alteration in function produced by any agent is coincident with changes in electric conductivity.

17. In the pathological specimens studied, active malignant growths have a high conductivity in comparison with adjacent normal tissue and the inactive portions of the same growth, and with growths of a non-malignant type.

GENERAL CONCLUSIONS

1. Influences which affect the general physical condition of the organism produce changes in electric conductivity in the dominating reactive tissues, these changes being uniformly and measurably manifested in the brain and the liver. Apparently these changes in conductivity appear more promptly than any gross clinical alteration.

2. Apparently the liver is more promptly and more markedly affected than any other tissue, as animals showing either no or very slight changes in the cerebrum and cerebellum will often show a marked alteration in the conductivity of the liver. On account of the wide variation in liver measurements and the apparent susceptibility of this organ to seasonal and environmental changes, the effects of applied agents are best determined by measurements of the cerebrum and cerebellum.

3. A study of the individual measurements from which the averages have been computed seems to indicate that the variations represent slightly different stages in a process that varies in rate in different animals and in the different organs of the same animal.

4. In view of the above indication and the direct evidence of the measurements we feel justified in the assumption that the first effect of stimulation within the organism is a slight decrease of the conductivity of the liver followed by a rapid continuous rise to above the normal as the state of exhaustion approaches; a slight and prompt increase in the conductivity of the cerebellum followed by a gradual continuous fall; a relatively slower increase in the conductivity of the cerebrum followed by a gradual continuous decrease.

5. These studies indicate that electric conductivity measurements provide a means whereby to further the interpretation of the normal operation of the organism, and whereby to measure the progress of pathological processes within the various organs and tissues.

6. From our findings to date, it would appear that the *intracellular changes in exhaustion and shock which are revealed by the microscope are paralleled by alterations in electric conductivity, and that both the histologic and the electric changes bear a direct relation to the vitality of the organ.*

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STUDIES ON THE EFFECT OF DIET ON THE WEIGHT OF THE HYPOPHYSIS AND THYROID GLAND OF THE ALBINO RAT, AND ON THE ACTION OF THEIR EXTRACTS ON THE ISOLATED SMALL INTESTINE

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This research was undertaken to determine whether alterations in weight of the thyroid and hypophysis and in the effects of their extracts on the isolated intestine could be brought about by different diets. In the previous studies made by Watson (13) and others, the observers were mainly concerned with the histological changes, and no study was made of the extracts from the glands. My conclusions are based on several series of observations. In the first series it was planned to test three diets characterized by oatmeal, vegetables and meat respectively. Later, in a second series, diets were included in which potassium iodide and "thyroxin" were administered to the test animals.

Material and methods. The rats were secured from the colony of The Wistar Institute. Two carefully selected litters of the same age were taken for each experiment. An equal number of rats from each litter was placed in the control and test cages, the sexes being kept separate. The ages and weights at the first feeding of the special diet were recorded. Weekly weighings were then made until the last animal had been killed. Tables 1 to 5 give in a condensed form the weights and other data.

At varying intervals during each experiment a rat from the test lot, with its accompanying control, was weighed, then killed by ether, measured, and the viscera removed. The weights of the thyroid, brain and hypophysis were taken separately and the percentage of water in the brain was determined. The thyroid and hypophysis were ground separately and extracted. The details of this last procedure will be given later.

The diets used: Oatmeal diet. The test diet was begun on September 30, 1919, in the form of steamed oatmeal and milk. The group com-

prised four females and six males, 27 days old. The animals throve and grew on this diet, although some cases of pneumonia appeared among both tests and controls. The experiment was continued from 105 to 117 days. Table 1 gives the data for this group.

Vegetable diet. This diet was begun on September 30, 1919. The rats seemed to be too young (27 days) for the test, as they showed symptoms of ill health. They were returned to the control diet on October 4 but on November 4 the test diet was resumed. This consisted of

TABLE 1

Oatmeal diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	3 T.	36	158	20.6	4.8	1.516	78.5	105	131
	3 C.	36	177	17.7	5.4	1.633	78.3	105	133
♀	2 T.	32	150	15.2	8.6	1.617	77.9	117	145
	2 C.	39	133	17.2	7.0	1.575	78.5	117	143

T = tests; C = controls.

TABLE 2

Vegetable diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	1 T.	113	228	22.6	6.5	1.734	*75.4	108	133
	2 C.	93	199	16.9	5.9	1.657	78.2	108	134
♀	4 T.	73	136	17.0	6.1	1.540	78.3	113	139
	4 C.	76	141	15.8	7.2	1.555	78.4	113	139

* This value is abnormal.

all green vegetables with occasional carrots, turnips, potatoes (steamed and raw), wheat, grape-fruit skins and beans. With the exception of a single male, all of the test rats were below the standard weight. Not only were they smaller in size, but were also obviously frail. They were less tame than the rats in other groups and toward the end of the experiment this reaction became even more noticeable (table 2).

Meat diet (a). This experiment was begun September 30, 1919, when the rats were 28 days old and had just been taken from the mother.

Steamed beef was fed twice a day. The rats became peeked, listless and inactive. They were cyanosed and cold to the touch. One rat was eaten by its brothers, despite the meat diet. On October 3 they were put on diet consisting of warm milk and condensed milk and wheat. On this they recovered, and were returned to the test diet on November 1. Raw beef was given from this date on, in preference to the steamed beef. Throughout the remaining period of the experiment, i.e., up to March 26, 1920, all were in splendid condition, always hungry, tame and

TABLE 3a

Series I. Meat

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	5 T.	24	226	19.4	7.7	1.836	78.2	125	185
	5 C.	23	232	17.0	7.0	1.837	78.0	125	186
♀	3 T.	24	169	18.2	9.2	1.771	78.2	109	168
	3 C.	24	165	17.6	9.6	1.737	78.3	109	169

TABLE 3b

Series II. Meat

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	3 T.	143	183	15.4	5.9	1.789	78.4	27	104
	3 C.	135	193	15.9	5.8	1.799	78.7	27	104
♀	3 T.	119	148	15.0	8.5	1.651	78.1	29	119
	3 C.	109	144	13.5	9.1	1.629	78.3	29	119

playful. The fur on the test animals was especially silky, soft and white, and the good condition of the group was striking. In three cases the lungs were found to be slightly infected (table 3 a).

A second series of observations was made to control this experiment with meat, and also to replace the first experiments made with potassium iodide and with "thyroxin," both of which had failed through accidents. The rats in these groups ranged from 76 to 98 days of age at the commencement of the test, and the experiments lasted from 14 to 37 days. Tables 3b, 4 and 5 give the data.

Meat diet (b). These rats were in excellent health; in two cases however the lungs were slightly infected. At each feeding they were given lean beef until entirely satisfied. Half of these rats were up to or above the standard weight (table 3 b).

Potassium iodide with normal diet. The first series tested has been omitted from the records because of serious lung infections and we have merely noted the effect of the lung infection on the water content of the brain.

In the second series a group was run for 26 to 32 days; the rats ranging in age from 83 to 120 days (table 4). The amount of KI given was 7 mgm. to 100 grams of body weight. In this group five of the twelve animals showed a light lung infection, but this was not severe enough to influence the percentage of water in the brain. The body

TABLE 4

Series II. Potassium iodide with normal diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	2 T.	144	174	15.0	6.0	1.781	78.4	26	120
	2 C.	137	202	19.4	6.7	1.833	78.7	26	120
♀	4 T.	119	137	15.0	7.8	1.772	78.1	32	115
	4 C.	115	147	13.9	8.2	1.793	78.3	32	115

weights were low. The health of the rats was good and no general effect of the KI was evident.

Thyroxin with normal diet. This material, first prepared by Doctor Kendall (5) and Kendall and Osterberg (6) was obtained from E. R. Squibb & Sons in the form of 0.2 mgm. tablets. One tablet was ground into fine powder and given each test rat at each feeding.

Each animal was kept in a separate cage in order to check the amount of food eaten. In the first group thus treated the controls failed to grow properly and the data have been discarded. A second group was therefore treated in the same manner. Table 5 gives the data for the second group. These rats remained in excellent health and more than half of the test rats were up to or above the standard weight. Two cases of slight lung infection were noted at autopsy (table 5).

Comments on weights. In all these groups (tables 1 to 5) the average body weights are low for the age in both the control and test animals,

and the weights of the organs are low for the body weights when compared with the standard values.

The discussion on the weights will be confined to a comparison of the tests with the controls for each sex, the age being the same and the animals compared being always members of the same litter. The data in the tables make twelve such comparisons possible.

As the weights of the thyroid and hypophysis are correlated with the body weight, it is necessary in comparing the weights for the test group with those for the controls to take the body weights into consideration. It is also necessary to keep in mind that the variability in the weights of these glands is high.

The procedure followed was to find from the appropriate table in *The Rat* (1) the percentage difference in the weights of the glands (thyroid and hypophysis) as there given according to the observed

TABLE 5

Series II. Thyroxin with normal diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	3 T.	133	187	17.4	6.0	1 863	78.1	27	111
	3 C.	129	224	16.1	6.2	1.877	78.3	27	111
♀	3 T.	138	169	13.8	8.5	1.793	78.6	28	118
	3 C.	128	169	15.5	9.0	1.727	78.5	28	118

body weights for each sex, and then to compare this with the corresponding percentage difference between the values for tests and controls in our records. If these two determinations agreed within 5 per cent, plus or minus, we concluded that the experimental conditions had not modified the weight of the organ.

When the data in tables 1 to 5 are treated in this manner there is some indication that in the "vegetable" group, table 2, the weight of the thyroid is increased and the weight of the hypophysis diminished, and in the "meat" group (tables 3a and 3b) that the weight of the thyroid is somewhat increased. In the other six pairs there is no difference shown by this method which can be attributed to diet. As the results are mainly negative, it is not deemed necessary to present the figures giving these relations.

For the body weights the brain weights are low. On the average the tests and the controls show nearly the same relative brain weight.

The dietetic treatment given these rats has not therefore modified the weight of the brain. When the percentage of water in the brain—on age—is compared in the tests with that in the controls, it is found to agree perfectly. Consequently the myelination process also has not been modified (2).

As previously noted, in a group of ten rats given potassium iodide for 60 days, all the animals were found to have severe lung infection and the data on weight were therefore excluded from the tables.

It was of interest to note however that in this group the percentage of water in the brain was 77.5 for the control rats and 77.6 for the tests. The percentage of water to be expected on age was 78.14 (1, table 74). Similarly, in the discarded group treated with thyroxin, but also suffering from severe lung infection, the percentage of water in the controls was 77.7 and in the tests 77.6. According to age 78.15 per cent was to be expected. The differences therefore are 0.6 and 0.5 per cent respectively.

These results agree with the observations of King (7) which show that severe lung infection in the mature rat reduces the percentage of water in the brain about 0.5 per cent.

Preparation of material for the study of the extracts. A test and control rat of the same sex were taken from a group and each weighed. The several organs were then removed and weighed. The thyroid, from which the parathyroids had not been removed, and the hypophysis were then ground separately with a small quantity of white Berkshire sand and mixed with Tyrode's solution. The extract thus obtained had a concentration of 0.25 per cent. The four test tubes containing the extracts from the thyroid and the hypophysis, test and control respectively, were put in the water bath (39°C.) for 2 hours.

An extra male rat was killed by crushing the cervical cord under ether, and a piece of the duodenal intestine was removed. This was placed immediately in a dish containing oxygenated Tyrode's solution and kept at 39°C. The intestinal strip was then cut lengthwise, freed from mesenteric tissue and a piece approximately 1.5 cm. prepared. This was attached at each end by passing through it a needle threaded with white silk, and suspended vertically in a glass tube filled with Tyrode's solution. The response of the strip could thus be recorded as described by Hatai and Hammett (4).

Action of extracts. In some cases after the strip had been immersed in Tyrode's solution the pulsations were for a time rapid and the tonus high. Occasionally it remained inactive. Usually therefore the in-

testine was left for 10 minutes in order that it might become normal in behavior. Sometimes it was necessary to initiate the pulsations by mechanical stimulation.

The general conditions which modify the response of the strip have been stated by Hatai and Hammett (4). One condition is sex and another is age. In accordance with the previous work, the observations here reported are only those made on the intestines of male rats 150 or more days of age.

The rat from which the intestine was taken had been kept without food for from 12 to 24 hours previous to the experiment. Before applying the extract the intestine was washed two or three times with the Tyrode's solution, and after returning to the normal beat the gland extract was added. Two cubic centimeters of the thyroid extract were used, but the hypophysis extract was added in proportion to the quantity on hand.

As pointed out and emphasized by Hammett and Tokuda (3) it is probably not the characteristic secretion of these glands in the extracts which causes the response of the intestinal strip, but something else.

The results according to diet may be summarized as follows:

Thyroid. For the extracts of the thyroid there was no clearly marked difference in the response to the test extract as compared with that to the control in the case of any of the diets here used.

Hypophysis. In the case of the extract from the hypophysis differences did occur as shown in charts 1 and 2.

Comments on the action of the extracts: Hypophysis extract. The control extract causes a relaxation of the strip in every case. This is sometimes preceded by a slight contraction (chart 1). The test extract in the oatmeal and vegetable diets causes a marked contraction (charts 1 and 2), but in the meat, the potassium iodide and the thyroxin groups, the reaction for the test extract is similar to that for the control (chart 3 for thyroxin).

It appears possible therefore that the effect of the extract of the hypophysis has been modified by the diet in the oatmeal and vegetable groups.

To obtain an explanation of this difference in behavior, some observations were made on the action of the glandular and nervous portions of the hypophysis taken separately.

In the first instance ten adult male rats were used, and the average body weight calculated. The rats were killed in the usual manner, the hypophysis removed, the ten glandular portions separated from the ten

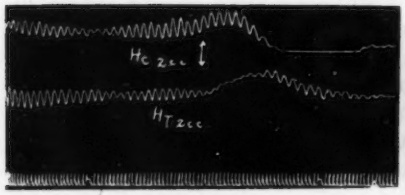

Chart 1

Chart 2

Chart 1. Oatmeal diet. Hypophyseal extract—From two female albino rats 144 days old. *H. C.* Control extract. *H. T.* Test extract.

Chart 2. Vegetable diet. Hypophyseal extract from two male albino rats 133 days old. *H. C.* Control extract. *H. T.* Test extract.

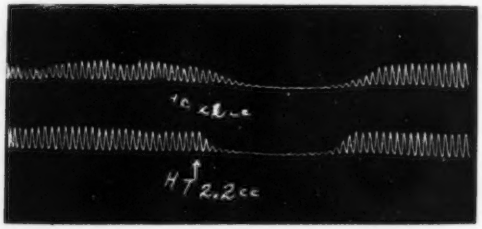

Chart 3. Standard diet with thyroxin (0.2 mgm. thyroxin). Hypophyseal extract from two male albino rats 97 days old. *H. C.* Control extract. *H. T.* Test extract.

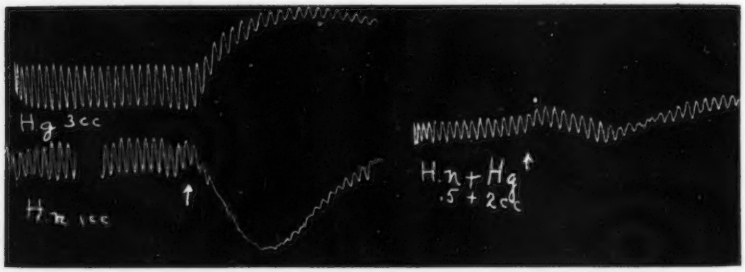

Chart 4 (a and b). Effects of extracts of parts of hypophysis—separately and combined, on the intestinal strip. (a) *H. g.* Extract of glandular portion alone. *H. n.* Extract of nervous portion alone. (b) *H. n.* and *H. g.* Combined in normal proportions.

nervous portions, and weighed separately. The values obtained are given in table 6. The nervous and glandular lobes were ground separately and the extracts tested on a piece of intestine from a male rat. The preparations were made in the morning and tested at 2 p.m. The amount of solution from the nervous portion was 5 cc. and that from the glandular 22.4 cc. According then to this ratio the extracts were tested on the intestine, first separately as nervous and glandular extracts, and then combined in the same proportion, as the extract from one rat. Chart 4 illustrates the results obtained. To determine any difference between the glands from males and females, ten females were taken and the glands treated in the same manner. The glandular and nervous portions, after being ground and extracted, were tested on the intestine from a *female* rat. The results were similar to those in chart 4, yet the curve from the nervous portion does not show as great a relaxation.

TABLE 6

Average weights of nervous and glandular lobes of hypophysis from rats used in tests

RATS	BODY WEIGHT	GLANDULAR	NERVOUS	TOTAL WEIGHT OF HYPOPHYSIS
	grams	*mgm.*	*mgm.*	*mgm.*
10♂	229	5.6	1.24	6.8
10♀	167	6.2	1.18	7.4
5♂	193	4.9	1.24	6.0
5♀	125	4.8	1.00	5.8

As a second check, five females and five males were treated as above, and the extracts tested first on a male intestine and then on female intestine to determine the differences in response. For the weights of these lobes see table 6.

The male and female glandular extracts gave similar results when tested on the male intestine, but the female nervous extract caused a greater relaxation than did the corresponding male extract. When the male and female glandular extracts were tested on female intestine, essentially similar results were obtained throughout.

Since the two parts of the hypophysis yield extracts which influence in opposite ways the intestinal strip, it is possible to interpret our results in the light of this relation.

In the normal reaction to the control hypophysis extract, the effect of the nervous portion predominates and the strip relaxes. When, on the other hand, the intestine contracts under the influence of the total

hypophysis extract, it means that the reaction characteristic for the glandular portion is predominating. At the moment, however, it is not possible to determine whether this predominance is due to an increase in the active substance characteristic for the glandular portion, or a decrease in that characteristic for the nervous portion of the gland.

DISCUSSION

There are several studies on these glands in rats and the results may be briefly considered according to the diet used.

Oatmeal. Watson (12) took six young rats, 6 weeks old, and fed them for from 4 to 8 weeks on a diet of uncooked oatmeal and water. An equal number of controls was fed bread and milk. He states that the thyroid was greatly enlarged and histologically gave evidence of greatly increased functional activity. A similar result had been obtained on an exclusive porridge diet made with skimmed milk (14). The average weights given by Watson in his first series are: test rats, thyroid 0.078 gram and controls, 0.029 gram.

As indicated in table 1, my test thyroids are on the average only very slightly heavier than the controls, thus showing much less difference than was obtained by Watson.

Vegetable diet. Slonaker (10) in his experiments with a vegetable diet on albino rats found the test rats to age more quickly, to be retarded in their growth, and on the whole frail and weak. The general appearance of my test rats checks with that of his series. Thus, with but one exception, a vigorous test male, all my test animals on this diet were considerably below the standard weight and in a poor physical state. Nevertheless the test thyroids appeared relatively somewhat heavier (table 2).

Meat diet. Watson (11) and Watson and Hunter (14), in experiments with an exclusive diet of ox flesh, found that the young rats did not do well, but with older rats particularly fine animals were obtained. Watson found in young rats on a meat diet distinct histological changes in the thyroid gland with wide variations in the amount of colloid and the secreting cells. These changes were frequent, but not constant, and were most pronounced in animals which did not gain normally in weight. As my tables 3a and 3b show, my general results were similar to those obtained by Watson.

Potassium iodide. Following intravenous injection of potassium iodide, Marine and Rogoff (9) found that iodine was taken up by the thyroids almost at once. The amount of absorption was correlated

with the functional state of the gland. So rapid was the absorption that practically none of the iodine was excreted. In my tests KI given with the food does not appear to produce any marked change in the size of the thyroid or the physiological effects of the extract. Kojima (8) notes, however, that administration of potassium iodide causes an accumulation of colloid material in the rat thyroid.

As to the effect of diet on the hypophysis, I do not find any observations which bear on the present problem.

SUMMARY

Feeding experiments with five diets were conducted on 200 albino rats, ranging in age from 27 to 250 days and fed for periods from 10 to 175 days upon *a*, oatmeal and milk; *b*, vegetables; *c*, meat; *d*, standard diet plus potassium iodide; and *e*, standard diet plus "thyroxin."

Observations were made on the effect of each diet on *a*, body weight; *b*, weights of the thyroid and hypophysis; and *c*, the physiological effect of the extracts of these glands on the isolated intestine. In addition, the action of the extracts of the parts of the hypophysis on the isolated intestine was determined and the effect of the diets on the brain weight and water content of the brain noted.

Body weight. The body weights in all the groups were low for the age in both control and test animals. There is but a slight difference in the final weights of the tests and controls. The controls were on the average heavier by 5 grams.

Weights of the glands. The normal variability in the weights of both the thyroid and hypophysis is large, and moreover these weights are correlated with the body weights. When compared with the standard tables, all the glandular weights were low for the body weights. Taking the averages, and recording the difference of the tests from the controls, the oatmeal thyroids were heavier than the controls by 1 mgm., the hypophysis by 2 mgm. In the vegetable group the thyroid was heavier by 4 mgm., a result mainly due to one unusually large male test rat. The hypophysis was lighter by 1 mgm. In the meat group the weight of the thyroid was somewhat greater, but that of the hypophysis not altered. In the potassium iodide and thyroxin groups there is practically no modification in the weights of the test glands.

Activity of the gland extracts: Thyroid. For the thyroid no definite difference in the effects of the control and test extracts could be made out in any of the diet groups.

Hypophysis. In the case of the hypophysis the test extract causes a contraction of the intestinal strip in the oatmeal and the vegetable diet groups. The control extract always causes relaxation.

Action of the extracts from parts of the hypophysis. The responses to the extracts from the nervous and glandular lobes of the hypophysis are opposed to each other. The glandular lobe extract causes a contraction and the nervous lobe extract a relaxation. The ratio of the weights of the nervous to the glandular lobes is 1 to 4 and when extracts from both lobes are combined in this proportion, the reaction obtained is intermediate or similar to that of the entire hypophysis. This suggests an explanation for the results found in the oatmeal and vegetable groups, in which for some reason the effect of the glandular portion predominated.

Brain weight and percentage of water. The brain weights are low for the body weights, but the relative weights in the tests and controls are similar. The several diets have not therefore modified the weight of the brain, and the percentage of water in the brains of the test and control series agrees perfectly. However, in two groups with severely infected lungs (groups not otherwise used) the percentage of water on age was low. This effect of lung disease on the percentage of water has been noted already by King (7).

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VARIATIONS IN OUTPUT OF BILE SALTS AND PIGMENTS DURING 24-HOUR PERIODS

Observations on Standard Bile Fistula Dogs

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In earlier communications from this laboratory (1), (2) have been reported many series of observations dealing with the physiology of the bile pigments and bile salts in healthy bile fistula dogs. It has been shown for example that the bile pigments can be modified at will by diet factors and many agencies which modify liver function (1). The bile pigment output therefore is varied by the constructive activity of the liver as well as by the commonly accepted disintegration of hemoglobin in the body. So too the bile acids or salts in bile fistula bile may be modified readily and promptly by diet factors (3). It is easy to demonstrate complete dissociation of these two activities of the liver showing that bile pigment production is a totally different function of the liver and separated completely from the bile salt production.

In all of our earlier experiments and as a routine we used unit daily collections of 6 hours. We felt that this period of 6 hours could be tolerated easily by a healthy dog and that the general condition of the dog was a very important element in the experiment. We have been able to maintain in perfect health many dogs on this daily routine. Some of our bile fistula animals have lived in perfect health for 2 to 4 years. We felt that the 6-hour unit collection made at the same time each day, preceded and followed by exercise, together with regular feeding hours, would be truly representative of the whole day's output. Many other workers have used 24-hour periods and we have reviewed this work elsewhere (4). In general these long collection periods tend to produce abnormalities in the dogs and the results may therefore be open to just criticism.

For our own information, we felt that it was necessary to make several 24-hour collection periods on our standard dogs. We believe these data

for 24-hour experiments are more satisfactory than any available in the literature. Moreover, it enables us to correlate our published work with some of the experiments of the older investigators. The routine given below does not cause impairment of health and was carried out with much care as to detail. The dogs were not upset by the procedure and were comfortable during the entire 24-hour period. Many other experiments of similar type were carried out but are not included here as the tabulated experiments are truly representative.

Methods. The method of bile fistula collection has been described elsewhere (4). So too the operative procedure and general hygienic routine have been reviewed (4). The *daily routine* for these experiments was as follows: The regular 6-hour collection was made for the first 4 days of the week. This daily routine consisted of yard exercise followed by a preliminary drainage period of 30 minutes which allowed the escape of any excess of night bile. In some dogs there is an accumulation of bile during the night owing to slight obstruction in the fistula when the collection tube is not in place. This causes more or less dilatation of the bile passages and is not a desirable factor. The regular 6-hour collection follows this preliminary drainage. Dogs are fed 2 hours after this collection is started unless note is made to the contrary. At the end of the collection, after the collection binders have been removed, the dogs are exercised, fed and locked in their cages.

On the fifth day of the week, the dogs were put through the regular routine, except that at 4 p.m., instead of taking off the binders, removing the drainage tubes and putting the dogs away in their cages for the night, a second collection period was started. This second period lasted from 4:00 to 10:00 p.m. At about 4:00 p.m., the dogs were transferred to their cages and were kept in an upright position by specially prepared night binders. These consisted of canvas slings suspended from the tops of the cages and provided with four holes for the dog's legs and an additional hole for the drainage tube. These binders were suspended at such a height that the dogs could either stand up, or rest the weight of their bodies on the binders by a slight flexion of their legs. Rests were provided for the dogs' heads, so that they could sleep comfortably in a semi-standing position. A third collection period extended from 10 p.m. to 4 a.m. on the following day and a fourth period lasted from 4 a.m. to 10 a.m., thus completing the 24-hour drainage and securing the day's output of bile in four 6-hour samples. The weekly routine thus consisted of a single 6-hour collection on the first four days of the week, followed by four consecutive 6-hour collections on the fifth day. The dogs were then rested till the following Monday.

Analysis of bile. The specimens of bile secured were analyzed for their content of bile acids and bile pigments. These methods have been fully described elsewhere in the publications of this laboratory. In brief, the bile acid method (5) consists in precipitation of proteins by alcohol, followed by an estimation of amino nitrogen present in the unhydrolyzed sample. A sample of the alcoholic filtrate is then hydrolyzed by means of sodium hydrate and its content of amino nitrogen estimated. This, minus the amount of amino nitrogen present in the unhydrolyzed specimen represents the amino nitrogen held in combination as taurocholic acid. The amount of taurocholic acid is estimated from the amino nitrogen by multiplying by the factor 36.72. Taurocholic acid is the only bile acid found in dog's bile.

The bile pigment method consists, in brief, of the following procedure. A 1 to 50 or weaker dilution of the bile in acid alcohol is filtered and allowed to stand in a flask for 24 hours, or until the pigment is converted to a blue green color. The solution is then read against a standard color wedge (4). The pigment readings are all expressed in terms of milligrams of bilirubin.

Feeding of dogs. The diet of the dogs, on the day of the experiments, was carefully controlled. At night the animals were always kept in metabolism cages, with only water before them. In the day time all food was excluded except the particular diet upon which the dogs were being kept, and they were fed at stated times twice a day. The amounts of food ingested were regularly recorded. Over the week-ends the dogs were rested on a mixed diet.

The carbohydrate diet referred to in the tables consisted of a mixture of potatoes, rice and milk, in the following proportions: potatoes, 40 per cent; rice, 28 per cent; milk, 32 per cent.

The meat diet consisted of meat alone. On most occasions extracted veal, obtained from the media kitchen, was used; but occasionally scraps of roast beef from the hospital kitchen were fed. On the days when mixed diet was used, the amounts ingested were not recorded. The mixed diet of course varied in content, but usually consisted of a mixture of bread and milk, table scraps and varying amounts of meat.

Experimental observations. These observations were made on three bile fistula dogs of very different type and are representative of the reaction of normal dogs under these conditions. One dog (20-99) was a very active, young, white bull dog, nervous and excitable but raised in the laboratory kennels and undisturbed by the laboratory routine. This dog had a strong habit spasm of its shoulder muscles. Bile

was completely excluded from the duodenum as shown by autopsy. Another dog (18-123) was a young adult mongrel, active and lively but good natured and not disturbed by the laboratory routine. Bile was completely excluded from the duodenum as shown by autopsy. The third dog (18-30) was a very strong, vigorous and rather vicious female of about 6 years of age. She was in perfect physical condition during the 2 years of experimental work. Her appetite was capricious and she refused many food mixtures. At operation the bile fistula was made as usual but the common duct only ligated, not cut. In due time, a small communicating channel was reëstablished permitting the flow of a little bile into the duodenum when external drainage was not in effect (at night and outside of collection periods). Autopsy showed the condition as described and recognized clinically.

Descriptions of bile fistula dogs. Following are the records of the essential points in the life histories of the dogs used in this work; also certain post-mortem findings.

Dog 20–99. White bull, young male. Weight on March 26, 1920, 32.5 pounds. Dog has marked nervous tic of both fore limbs. Operation on March 26, 1920—common duct cut between ligatures; fistula made as usual. Recovery speedy and uneventful. Dog set up on April 5th for daily bile collections. Used in bile metabolism work from this time until January 29, 1921. Stools clay colored. January 26, 1921, blood serum quite icteric. Red cell hematocrit, 38 per cent. On January 31st, dog was observed to be very feeble and collections of bile were discontinued. Dog went rapidly downhill and on February 8th began to bleed from his fistula. Dog bled all night and in morning red cell hematocrit was 33 per cent, serum being decidedly icteric. Dog given excess of chloroform and immediately autopsied.

Autopsy findings: Remains of an emaciated dog (weight 20 lbs.). Fistula appears normal externally, but a bloody bile is exuding from it. Gums and other mucous membranes are extremely anemic. Median incision reveals normal subcutaneous tissues and muscles. There are a few fibrous adhesions between the omentum and operative site. The intestine and pancreas are stained a dark, maple sugar color. The costo-chondral junctures are enlarged. The ribs are very fragile and almost completely destitute of lime salts. Almost all the ribs show fusiform enlargements where spontaneous fractures with subsequent healing have occurred. The flat bones show the same process. The long bones of the extremities seem normal and contain normal marrow. The bones of the skull show some diminution of lime salts.

Heart and lungs—negative. Thyroid—small and pale. Spleen—negative.

Liver appears normal. No cirrhotic thickening. Slight scarring on a portion of ventral surface. Gall passages—hepatic and cystic ducts are patent and moderately dilated. Common duct is completely occluded and ends in a blind pouch.

Pancreas—deep, maple sugar color. Negative, on section. Duct opens normally into duodenum. Stomach and intestines—negative except for brown color

of muscle coats. Kidneys and bladder and prostate—negative. Adrenals—negative. Lymph nodes—a few are enlarged in region of liver hilus, also a few of the mesenteric nodes are enlarged. Brain appears negative superficially.

Microscopical sections: Liver—slight degree of round cell infiltration around some of smallest ducts. No cirrhosis. Moderate degree of brown atrophy. Central cells pale and contain brown pigment. A study of the bone abnormalities will be reserved for a future communication.

Dog 18-80. White bull, middle age, female. Weight on August 19, 1919, 39 pounds. Operation on August 19, 1919. Three ligatures placed on duct, but duct not cut. Fistula as usual. Recovery uneventful. Dog set up on September 10th. November, 1920, feces gave positive test for stercobilin with Schlesinger's reagent, indicating that exclusion of bile from duodenum is incomplete. Dog keeps her weight well. June 15, 1921, dog normal. Chloroform anesthesia and killed; autopsy performed at once.

Autopsy findings: Heart—normal. Lungs—slight anthracosis, normal on section. Spleen—rather large, firm and fibrous on section.

Liver—normal size, red-brown in color with occasional yellowish tinge; lobulation normal on section. Gall bladder attached to abdominal wall by way of operative fistula. A fine probe can be passed from papilla into common duct. Site of operation shows a definite stricture leaving an aperture not over 1 mm. in diameter. A slight dilatation exists above this. Probably drainage into duodenum was pretty efficient at night.

Stomach and intestines—show congestion due to a toxic proteose administered a few days previously. Pancreas—quite firm; section normal. Kidneys—fairly large in size, capsule adherent; section normal. Bladder—normal. Skeletal muscle—normal; slight amount of fat. Bones—apparently no thinning and no loss of lime salts. Bone marrow—no hyperplasia; normal in appearance.

Dog 18-123. White and tan, adult, female. Weight on October 9, 1919, 25 pounds. October 9th, exposure to x-rays over spleen region (200 milliampere minutes at distance of 10 inches from skin). Operation on October 21, 1919. Weight 26 pounds. Common duct cut and ligated above and below. Fistula as usual. Recovery uneventful. Dog set up on October 31st. Stools clay colored. Used for daily collections from this time until March 8, 1921, when she became intoxicated. Died March 10th.

Autopsy findings: Remains of an emaciated dog. Fistula externally appears in normal condition. Abdomen is moderately distended. Median incision reveals lack of subcutaneous fat. The ribs are quite fragile, containing a very small amount of lime salts. They bear numerous greyish enlargements, at the sites of previously healed fractures. The lower end of the sternum and the adjacent costal cartilages are bulged forward.

Heart and lungs—negative. Liver—a few small scars are present over the surface of the organ. It is negative on section. The larger ducts are much distended. Common duct is completely occluded at operative site. Spleen—small ($12 \times 3 \times 0.5$ cm.); weight—11 grams. On section, the fibrous elements are seen to be increased in amount. Kidneys—pale in color; capsules strip readily. Over the surfaces are a few small retention cysts. Pelves—negative. Adrenals—negative. Pancreas—brown in color; negative, on section. Intestines—brown color in muscle coats, moderate distention with gas.

Microscopical sections: Liver—central cells of lobules show beginning disintegration. Their nuclei show some degree of pyknosis, while cytoplasm is very pale and reticulated. Some of these cells contain masses of brown material. Peripheral cells of lobule—normal in appearance. No cirrhotic changes. No cholangitis. Spleen—capsule and trabeculae thickened. Malpighian bodies normal. Sinusoids widely patent and with red cells in them. Pulp cells appear normal. There is probably a slight relative decrease in parenchyma cells.

Table 1 gives three similar experimental periods on the same dog. This bile fistula animal had been under observation on the standard laboratory routine for about 13 months. The only complicating detail was an exposure of the splenic area to a considerable x-ray dose 2 weeks before the fistula operation. This dog shows the fluctuation in bile salts which are observed and so far are unexplained. We note the rise in bile salts following a change from a carbohydrate to a meat diet in the first and second experiments. This certainly is a physiological constant. The 24-hour collections show a moderately uniform series. There are fluctuations as are observed in the daily 6-hour periods but in general the four periods of 6 hours each in the 24-hour unit show uniform figures for volume and bile salts. We can detect no difference in this dog between the day and night collection periods. The bile pigment figures in this dog are high—in fact, more than twice normal and we observe some remarkable fluctuation in values. We cannot explain these reactions but they are more frequent in dogs with splenectomy as reported elsewhere (6).

Table 2 shows two experimental periods on the same dog. We note no difference during the night and day periods of the 24-hour collections. The general picture is very much like that noted in table 1.

Table 3 shows two more meat feeding experiments on the third dog of this series. The general type of reaction is the same as in the other two dogs. The rise in bile acids when the dog changes from a carbohydrate to a meat diet is very pronounced and probably more conspicuous in this dog which always ate sparingly of the carbohydrate mixtures but largely of the meat diet. During the second 24-hour period, the dog struggled very vigorously in an attempt to get out of the binder, but to no effect. There is a rise in bile volume flow but little other change to be attributed to this over-activity. As a rule during the night and much of the day, this dog dozed comfortably in its binder. There are very great fluctuations in bile pigment values.

Table 4 shows three collection periods on the same dog on a carbohydrate diet. There is a fairly uniform level of bile acid excretion

TABLE 1

Twenty-four hour bile collections after meat feeding

Dog 18–123. Brindle, mongrel, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAURO-CHOLIC ACID IN 6 HOURS	BILI-RUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
9/27	26	0.266	6.92	254	50.6	18.0	Mixed diet
9/28	30	0.269	8.07	296	32.9	19.0	Carbohydrate diet
9/29	38	0.324	12.31	452	25.2	18.8	Carbohydrate diet
9/30	32	0.352	11.26	413	37.8	18.0	Beef scraps (430 gm.)
10/1 a	40	0.390	15.60	572	50.2	18.3	Beef scraps (1000 gm.) 10 a.m.–4 p.m.
10/1 b	43	0.321	13.07	479	61.0		Dog vomited mucus 4 p.m.–10 p.m.
10/2 c	38	0.460	17.48	642	40.3		Dog vomited mucus 10 p.m.–4 a.m.
10/2 d	45	0.348	15.66	574	51.7		4 a.m.–10 a.m.
11/15	29	0.112	3.25	119	37.9	17.5	Carbohydrate diet (1000 gm.)
11/16	22	0.180	3.96	145	25.3	17.5	Carbohydrate diet (500 gm.)
11/17	32	0.318	10.18	374	33.4	17.0	Meat diet (600 gm.)
11/18 a	49	0.443	21.71	797	32.8	17.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
11/18 b	37	0.255	9.43	344	15.7		4 p.m.–10 p.m.
11/19 c	40	0.398	15.92	584	17.9		10 p.m.–4 a.m.
11/19 d	21	0.417	18.76	688	98.6		4 a.m.–10 a.m.
11/29	35	0.439	15.36	564	42.4	18.5	Carbohydrate diet (870 gm.)
11/30	28	0.595	16.66	611	29.7	17.5	Carbohydrate diet (700 gm.)
12/1	27	0.308	8.31	303	30.6	17.0	Meat diet (350 gm.)
12/2	32	0.397	12.70	466	54.7	16.0	Meat diet (600 gm.)
12/3 a	45	0.496	22.32	818	45.4	16.5	Meat diet (425 gm.) 10 a.m.–4 p.m.
12/3 b	45	0.368	16.56	607	39.5		4 p.m.–10 p.m.
12/4 c							10 p.m.–4 a.m.
12/4 d	27	0.410	11.07	406	45.1		4 a.m.–10 a.m.

during most of the collection periods. In two of the observation periods there is a fall in bile salt output in the early morning hours. This may or may not be associated with a fall in volume output. The bile pigment output is very irregular and periods of semi-obstruction in the bile passages may have been responsible.

TABLE 2

Twenty-four hour bile collections after meat feeding

Dog 20–99. White bull dog, young, adult male

DATE, 1920	VOLUME	AMINO NITROGEN		TAURO-CHOLIC ACID IN 6 HOURS	BILI-RUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/29	37	0.411	15.21	558	56.7	28.0	Carbohydrate diet (975 gm.)
11/30	46	0.170	7.82	286	54.0	26.0	Carbohydrate diet (700 gm.)
12/1	35	0.280	9.80	358	34.1	25.5	Meat diet (800 gm.)
12/2	50	0.326	16.30	598	22.1	25.0	Meat diet (600 gm.)
12/3 a	59	0.354	20.89	766	36.3	24.8	Meat diet (450 gm.) 10 a.m.–4 p.m.
12/3 b	50	0.283	14.15	519	47.0		4 p.m.–10 p.m.
12/4 c	48	0.198	9.50	349	63.0		10 p.m.–7 a.m.
12/4 d	40	0.382	15.28	561	33.4		7 a.m.–1 p.m.
11/15	30	0.267	8.01	292	35.5	26.0	Carbohydrate diet (1000 gm.)
11/16	51	0.194	9.89	361	65.0	26.0	Carbohydrate diet (500 gm.)
11/17	53	0.165	8.74	319	25.8	25.8	Meat diet (600 gm.)
11/18							Meat diet
11/19 a	25	0.318	7.95	290		26.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
11/19 b	21	0.141	2.96	108			4 p.m.–10 p.m.
11/20 c	38	0.142	5.40	197			10 p.m.–4 a.m.
11/20 d	36	0.172	6.20	226			4 a.m.–10 a.m.

Table 5 shows a series of three observations on another dog receiving a carbohydrate diet. This dog shows a fairly uniform output of bile pigment. The bile salt output is not decreased during the night hours.

Table 6 is of considerable interest and shows a dog with incomplete bile fistula on a carbohydrate diet. At night, when this dog's fistula was closed, there could flow into the duodenum a certain amount of bile. This bile influx increased the bile acid flow of the following morn-

ing. This fact, established at autopsy, we believe explains at least in part the fall in bile salts noted in both 24-hour periods. This fall in bile salts is progressive after the first 6-hour collection and reaches its lowest level in the fourth collection period in the 24-hour experiment. The reaction therefore is much like that following an ingestion of bile in the early morning (refer to table 7). The bile pigment output is

TABLE 3

Twenty-four hour bile collections after meat feeding

Dog 18–30. Bull dog, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours.				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/15	15	0.448	6.72	247	87.7	34.0	Carbohydrate diet (170 gm.)
11/16	11	0.638	7.02	258	62.0	33.5	Carbohydrate diet (225 gm.)
11/17	17	0.971	16.51	606	85.3	33.5	Meat diet (600 gm.)
11/18 a	26	0.830	21.58	792	99.1	34.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
b	9	0.770	6.93	254	10.7		4 p.m.–10 p.m.
11/19 c	11	0.908	9.99	367	9.2		10 p.m.–4 a.m.
d	17	0.948	16.12	592	23.3		4 a.m.–10 a.m.
11/29	9	1.500	13.50	495	72.4	36.0	Carbohydrate diet (450 gm.)
11/30	8	1.120	8.95	329	102.0	34.5	Carbohydrate diet (450 gm.)
12/1	16	0.644	10.30	378	116.8	35.0	Meat diet (800 gm.)
12/2	30	0.738	22.14	810	41.8	35.0	Meat diet (600 gm.)
12/3 a	15	0.850	12.75	468	43.3	35.0	Meat diet (450 gm.) 10 a.m.–4 p.m.
b	32	0.549	17.57	644	21.2		Dog struggling 4 p.m.–10 p.m.
12/4 c	22	0.595	13.09	480	16.9		10 p.m.–4 a.m.
d	17	0.877	14.91	547	111.3		4 a.m.–10 a.m.

high but pretty uniform. The volume output in this dog is constantly subnormal as compared with the average animal.

Table 7 gives the results of three experiments on different bile fistula dogs, given large doses of taurocholic acid (2 grams) in solution by stomach. The reaction is remarkably uniform in these three different animals. The great cholagogue action does not modify the bile pigment

TABLE 4

Twenty-four hour bile collections after carbohydrate feeding

Dog 20–99. White bull dog, adult, male

DATE, 1920	VOLUME	AMINO NITROGEN		TAURO-CHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
10/11	36	0.253	9.11	333	25.1	27.0	Mixed diet
10/12	44	0.283	12.45	457	21.4	26.3	Mixed diet
10/13	11	0.224	2.46	90	2.9	26.8	Carbohydrate diet (650 gm.)
10/14	63	0.112	7.05	258	20.5	25.5	Carbohydrate diet (1000 gm.)
10/15 a	23	0.140	3.22	118	15.1	25.0	Carbohydrate diet (1000 gm.) 10 a.m.–4 p.m.
10/15 b	54	0.211	11.39	418	30.1		4 p.m.–10 p.m.
10/16 c	17	0.407	6.92	253	21.3		10 p.m.–4 a.m.
10/16 d	21	0.309	6.49	237	31.4		4 a.m.–10 a.m.
11/8	47	0.438	20.58	755	58.9	27.8	Mixed diet (1150 gm.)
11/9	57	0.397	22.62	830	117.0	27.5	Carbohydrate diet (1000 gm.)
11/10	45	0.295	13.27	487	88.5	27.3	Carbohydrate diet (1000 gm.)
11/11	49	0.337	16.50	605	112.7		Carbohydrate diet (1000 gm.)
11/12 a	38	0.308	11.70	429	83.2	26.3	Carbohydrate diet (700 gm.) 10 a.m.–4 p.m.
11/12 b	30	0.322	9.66	353	61.6		4 p.m.–10 p.m.
11/13 c	36	0.280	10.08	370	72.7		10 p.m.–4 a.m.
11/13 d	27	0.364	9.82	355	72.4		4 a.m.–10 a.m.
12/6	55	0.255	14.02	514	15.3	24.5	Carbohydrate diet (1000 gm.)
12/7	40	0.312	12.48	457	62.4	24.5	Carbohydrate diet (700 gm.)
12/8	40	0.326	13.04	478	89.8	24.0	Carbohydrate diet (1000 gm.)
12/9	41	0.252	10.33	379	57.0	23.5	Carbohydrate diet (1000 gm.)
12/10 a	38	0.227	8.62	314	50.8	23.5	Carbohydrate diet (1000 gm.) 10 a.m.–4 p.m.
12/10 b	27	0.241	6.51	238	38.8		4 p.m.–10 p.m.
12/11 c	37	0.113	4.18	153	40.3		10 p.m.–4 a.m.
12/11 d	28	0.284	7.95	290	53.0		4 a.m.–10 a.m.

TABLE 5

Twenty-four hour bile collections after carbohydrate feeding

Dog 18–123. Brindle mongrel, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAURO-CHOLIC ACID IN 6 HOURS	BILI-RUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
10/11	31	0.352	10.91	400	41.4	18.0	Mixed diet
10/12	26	0.297	7.72	282	22.6	17.0	Mixed diet
10/13	48	0.322	15.45	568	20.9	18.0	Carbohydrate diet (620 gm.)
10/14	32	0.154	4.93	180	24.5	17.0	Carbohydrate diet (830 gm.)
10/15 a	18	0.267	4.81	176	27.2	16.5	Carbohydrate diet (710 gm.) 10 a.m.–4 p.m.
10/15 b	40	0.168	6.72	245	27.8		4 p.m.–10 p.m.
10/16 c	14	0.225	3.15	115	15.3		10 p.m.–4 a.m.
10/16 d	5				6.1		4 a.m.–10 a.m.
11/8	29	0.297	8.61	314	47.4	17.0	Mixed diet
11/9	23	0.340	7.82	286	36.0	17.0	Carbohydrate diet (750 gm.)
11/10	30	0.295	8.86	324	33.7	17.5	Carbohydrate diet (1000 gm.)
11/11	38	0.309	11.75	432	32.2		Carbohydrate diet (860 gm.)
11/12 a	36	0.280	10.08	370	34.3	17.5	Carbohydrate diet (650 gm.) 10 a.m.–4 p.m.
11/12 b							4 p.m.–10 p.m.
11/13 c	23	0.322	7.41	271	50.0		10 p.m.–4 a.m.
11/13 d	22	0.392	8.62	351	53.6		4 a.m.–10 a.m.
12/6	36	0.255	9.18	337	117.9	16.3	Carbohydrate diet (800 gm.)
12/7	25	0.071	1.77	65	37.0	16.5	Carbohydrate diet (675 gm.)
12/8	23	0.298	6.85	251	36.0	16.5	Carbohydrate diet (730 gm.)
12/9	26	0.098	2.54	93	36.2	16.5	Carbohydrate diet (800 gm.)
12/10 a	24	0.156	3.74	137	25.5	16.5	Carbohydrate diet (1000 gm.) 10 a.m.–4 p.m.
12/10 b	36	0.156	5.61	206	35.1		4 p.m.–10 p.m.
12/11 c	21	0.129	2.81	103	22.3		10 p.m.–4 a.m.
12/11 d							4 a.m.–10 a.m.

output. The greater part of the 2 grams of taurocholic acid is eliminated in the 6 hours following its ingestion. In spite of this, the cholagogue action is much in evidence in the second period of 6 hours. This period of cholagogue action without any excess of bile salt excretion shows that the outpouring of an excess of fluid in bile is not a simple reaction to preserve a certain salt concentration in the bile passages.

TABLE 6

Twenty-four hour bile collections after carbohydrate feeding

Dog 18–30. Bull dog, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/8	6	1.170	7.02	257	51.2	35.8	Mixed diet (800 gm.)
11/9	8	0.964	7.71	283	69.6	35.0	Carbohydrate diet (70 gm.)
11/10	4	1.475	5.90	216		34.5	Carbohydrate diet (210 gm.)
11/11	10	0.857	8.57	314	100.0	34.0	Carbohydrate diet (230 gm.)
11/12 a	9	1.025	9.22	338	98.3	33.5	Carbohydrate diet (220 gm.) 10 a.m.–4 p.m.
11/12 b	7	0.616	4.31	158	85.1		4 p.m.–10 p.m.
11/13 c	10	0.364	3.64	134	96.0		10 p.m.–4 a.m.
11/13 d	6	0.420	2.52	92	71.9		4 a.m.–10 a.m.
12/6						35.0	Carbohydrate diet (50 gm.)
12/7	8	1.690	13.52	496	84.6	34.5	Carbohydrate diet (200 gm.)
12/8	10	1.090	10.90	400	98.3	34.0	Carbohydrate diet (370 gm.)
12/9	11	0.882	9.70	356	110.3	33.5	Carbohydrate diet (475 gm.)
12/10 a	22	0.383	8.43	309	51.3		Carbohydrate diet (490 gm.) 10 a.m.–4 p.m.
12/10 b	17	0.412	7.00	257	76.2		4 p.m.–10 p.m.
12/11 c	5	0.925	4.62	169	88.4		10 p.m.–4 a.m.
12/11 d	5	0.570	2.85	105	146.0		4 a.m.–10 a.m.

This emphasizes the ease with which the various elements of bile secretion may be dissociated—the fluid and salt elements and bile pigments. This all speaks for a remarkable diversity in functional activity of the liver cell. It may be well to note that this crude solution of taurocholic acid was obtained from whole dog's bile evaporated with charcoal to a soft paste, extracted with water and filtered. Obviously other elements besides taurocholic acid are present.

TABLE 7

Twenty-four hour bile collections after bile feeding

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
Dog 18–123. Brindle, mongrel, adult, female							
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
12/13	38	0.410	15.58	572	40.7	16.5	Mixed diet
12/14	38	0.314	11.93	440	33.3	16.5	Meat diet
12/15	40	0.422	16.88	620	34.8	17.0	Meat diet
12/16	58	0.434	25.17	924	33.7	17.0	Mixed diet
12/17 a	27	0.412	11.12	408	26.9	16.8	Mixed diet 10 a.m.–4 p.m.
12/17 b	70	0.909	63.63	2335	18.3		*4 p.m.–10 p.m.
12/18 c	36	0.369	13.88	509	22.7		10 p.m.–4 a.m.
12/18 d	18	0.468	8.42	309	24.7		4 a.m.–10 a.m.
Dog 18–30. Bull dog, adult, female							
12/13	13	0.452	5.87	215	61.5	33.5	Mixed diet
12/14	16	0.814	13.02	478	80.2	34.0	Mixed diet
12/15	13	1.100	14.30	525		34.0	Mixed diet
12/16	14	1.428	19.99	733	61.5	33.5	Mixed diet
12/17 a	7	0.800	5.60	206	43.9	33.3	Mixed diet 10 a.m.–4 p.m.
12/17 b	60	1.250	75.00	2755	67.2		*4 p.m.–10 p.m.
12/18 c	11	1.010	11.11	408	46.0		10 p.m.–4 a.m.
12/18 d	8	1.190	9.52	349	52.3		4 a.m.–10 a.m.
Dog 20–99 White bull dog, young adult, male							
12/13	40	0.254	10.16	373	33.4	23.5	Mixed diet
12/14	48	0.285	13.68	502	28.4	24.0	Mixed diet
12/15	50	0.183	9.15	334	25.0	24.0	Mixed diet
12/16	50	0.238	11.90	437	41.8	23.5	Mixed diet
12/17 a	32	0.337	10.78	396	41.8	23.3	Mixed diet 10 a.m.–4 p.m.
12/17 b	67	0.955	63.98	2347	36.5		*4 p.m.–10 p.m.
12/18 c	63	0.211	13.29	487	35.6		10 p.m.–4 a.m.
12/18 d	45	0.211	9.45	345	32.9		4 a.m.–10 a.m.

* At 4 p.m., a solution of extracted dog's bile, containing 2 grams of taurocholic acid, made up to 400 cc. with water, was given by stomach tube.

DISCUSSION

In all experiments with bile fistula dogs we believe it essential to work with animals of two types—one with complete exclusion of bile from the duodenum and one with incomplete exclusion or a little seepage of bile through a small strictured common duct. This second type is well illustrated above (dog 18-30) and is of much importance. These dogs during drainage periods will certainly yield a complete bile flow as the flow through the stricture is not easy and in the standing posture the escape of bile into the cannula is very prompt and complete. But during resting or night periods there is a flow of an undetermined volume of bile into the duodenum and this is sufficient to maintain these dogs in perfect physical condition. There are certain objections to both types of experimental animal but a combination of these types gives data which we believe are trustworthy and of real physiological significance.

Dogs with biliary fistulas and complete exclusion of bile from the duodenum suffer from a variety of peculiar intoxications and metabolic disturbances, some of which have been noted previously. We record certain bony changes in the autopsy notes of two of the bile fistula dogs. (Dogs 20-99 and 18-123.) These abnormalities are of considerable interest as relating to the metabolism of inorganic salts and will be the subject of a future communication.

Our experiments with the long 24-hour collections give no constant results to indicate a decided fall in output at night or during the early morning hours. The amounts of bile acids and bile pigments are not uniformly decreased during the periods of sleep. The same statement applies to volume output of bile.

We wish again to point out the ease with which one can dissociate the various constituents of the bile (table 7). Large doses of crude taurocholic acid cause a prompt rise in bile salt excretion so that practically all the taurocholic acid is excreted within 6 hours after its ingestion. But the cholagogue action persists during the second 6-hour period or longer, showing dissociation of salt and fluid elimination. The bile pigment output remains constant throughout.

One can detect no difference in cholagogue action whether the bile salt is given during the morning or evening. The reaction will be the same during the day or during night periods when the dog is sleeping comfortably most of the time.

SUMMARY

Bile fistula dogs will show little if any difference in the output of bile, bile salts or bile pigments during four consecutive 6-hour periods. There are no constant differences to be made out. If anything can be said, it is to the effect that at times the night output is slightly less than the normal daily output. This applies to healthy bile fistula dogs.

Peculiar bony abnormalities are noted as related to the constant loss of bile in such animals.

Complete dissociation of the bile constituents can be demonstrated (table 7) after a large dose of taurocholic acid. Following a 2-gram dose we observe a great rise in bile salt content during the first 6-hour period. During the second 6-hour period the bile salt content is about normal but the cholagogue action is still much in evidence and the fluid output therefore is dissociated from the bile salts. The bile pigments are constant before and after the period of bile salt administration and elimination.

This all speaks for a remarkable diversity in liver cell function—a point not too frequently emphasized.

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THE RELATIONSHIP BETWEEN NERVOUS AND HORMONE CONTROL OF THE RESPIRATORY CENTER

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The changes which can be brought about in the activity of the respiratory center in laboratory animals by various forms of sensory stimulation and by alterations in the composition of the blood are often decidedly variable and this is the case not only for animals of different species but also, not infrequently, for individuals of the same species. There can be no doubt that a part of this inconstancy in results is dependent on the fact that the observations have usually been made on animals that are under varying degrees of anesthesia. The degree of anesthesia affects the respiratory center in part because this is directly depressed by the anesthetic and, in part, because of dulling of the sensitivity of the higher (affective) centers which, it is well known, have a close relationship in the conscious animal with changes in respiration. Thus, in a conscious animal even the mildest stimulation of the pain receptors is usually associated with a decided change in breathing, which is not always the case under conditions of general anesthesia.

In order to eliminate the errors due to unequal degrees of anesthesia we have chosen, for investigation of the behavior of the respiratory center, animals in which the higher centers have been removed by section of the brain stem about the level of the anterior corpora quadrigemina. In certain animals, notably the cat, this operation usually furnishes a preparation in which the breathing proceeds with more or less regularity for many hours and, if the decerebration be quickly performed immediately after placing the animal deeply under ether, every trace of the anesthetic is removed from the blood within an hour after the decerebration. As has been pointed out elsewhere (1) many cats do not respire normally after the decerebration, a condition of hyperpnea becoming gradually developed for the incidence of which the exact position of the section of the brain stem and the age of the animal are the chief determining factors.

In a successful decerebrate preparation the chief respiratory center is isolated from two types of impulses which in the intact animal greatly influence its activity, namely, those from the higher (cerebral) centers and those from the nasal mucosa. Although this partial isolation must be borne in mind in the interpretation of the results obtained by experimental alterations in the activity of the center, many of these are of so definite and constant a nature that they must be considered as representing conditions which come into play in the intact animal.

The observations recorded in the present paper have been made on animals (mainly cats) in which the breathing was perfectly regular one hour after decerebration by Sherrington's method. To prevent fall in body temperature the preparations were kept on a heated table with the head end somewhat raised on a hot water bag. The rectal temperature was frequently observed and the heating suitably adjusted if any changes in this were observed to occur.

Several related problems have been investigated all bearing on the general question of the excitability of the center during alterations in the composition of the blood, or after removal of the afferent impulses arriving at it through the vagus nerves. The results are grouped under various headings.

THE EFFECT PRODUCED ON BREATHING BY SECTION OF THE VAGUS NERVES. It is usually stated that the action of the respiratory center besides becoming very slow also becomes irregular and inadequate when the vagus nerves are cut in animals from which the higher centers have been removed (2). We have found this to be the case in decerebrate rabbits but not, as a rule, in decerebrate cats. Decerebration in animals of the former group is not generally so successful as in those of the latter and it is best performed by means of a blunt hook passed into the brain case through a trephine hole. Only a few of the decerebrations are really successful in the sense that regular and efficient breathing persists after the operation, but when it does so, section of both vagi invariably causes a complete breakdown and the rabbit soon dies of asphyxia. In some rabbits section of one vagus nerve is sufficient to have this effect, as is illustrated in the tracing of figure 1. In this case section of the right vagus was immediately followed by a prolonged period of apnea succeeded by very slow deep respirations. At the points indicated on the tracing small amounts of an approximately normal solution of hydrochloric acid were injected intravenously with the result that the breathing became deeper without any change in rhythm.

In decerebrate *cats* section of one vagus does not usually have any effect on breathing but section of the nerve on both sides causes the rhythm to decline and the depth to become greater. In the experiment shown in figure 2, the upper tracing is that from a tambour connected with the thorax and the lower, from a Gad-Krogh spirometer placed in series in a closed system of wide-bore tubing furnished with valves and soda-lime absorbers and connected with the trachea. The volume of air breathed in a unit of time is therefore determined by multiplying the height of each respiration by the rate. The air in the closed system

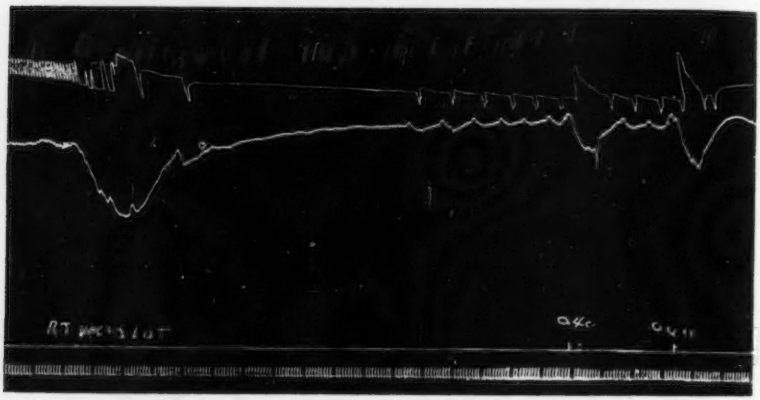

Fig. 1. (Rabbit 4) Cutting of vagus nerve on right side in decerebrated rabbit caused entire break down of respiratory rhythm (upper tracing) and temporary fall in blood pressure (lower tracing). The respirations were recorded by a balloon placed between the liver and diaphragm. The effect of injection into the peripheral end of one of the carotid arteries of 0.4 cc. of weak HCL in 0.9 NaCl is also shown. Time in seconds

contained excess of oxygen. By comparison of the volume of air breathed per minute it will be observed that a decrease occurred immediately after vagotomy as in the experiment shown, where it decreased from 960 cc. to 700 cc. per minute. In such cases pulmonary ventilation is adequately performed with a lesser minute volume of air because each breath is deeper and therefore more thoroughly renews the air in the alveoli. The difference in minute volume is however not much changed as a rule and sometimes it may even be slightly increased and it is interesting that when progressively greater percentages of CO_2 are inspired it increases at about the same rate before as after vagotomy

Fig. 2. (Cat 29) Cutting of both vagi in decerebrated cat caused the respirations to change from 45 to 15 and the volume from 960 cc. to 700 cc. per minute. Upper tracing tambour, lower, Krogh spirometer. Both before and after vagotomy, the breathing progressively increased during the periods of registration. Time in seconds.

(curve I, fig. 4). There were no signs of asphyxia in these animals after vagotomy—as judged from the behavior of the blood pressure, the longevity of the animal and the arterial character of the blood—and the oxygen consumption remains normal—as judged from the slope of the descent of the spirometer curve.

It is of interest to compare the rate of breathing before and after the vagotomy, as is done in the following table:

Rate of breathing

NUMBER	BEFORE VAGOTOMY	AFTER VAGOTOMY	RATIO
	per minute	*per minute*	
II	46	16	2.87:1
III (fig. 3)	41	20	2.05:1
Ia	42	14	3.00:1
IIIa	23	11	2.09:1

The fact comes to light that there is often a simple ratio between the rate before and after vagotomy. What this may mean is difficult to say. It suggests that there is a certain fundamental rhythm of the center which is independent of afferent vagal impluses and in the decerebrate animal at least is influenced mainly by the temperature of the blood and perhaps by extreme anoxemia and by poisons. It will be observed that this rhythm varied between 11 and 20 in our experiments, these differences being probably partly dependent on differences in temperature of the animal. Although this was observed regularly so as to indicate what degree of heat we should turn on to the table, it might vary by 1°C. and it is altogether likely that the temperature of the blood in the medulla would vary still more. This fundamental rhythm is accelerated 2 or 3 times by the vagal impulses but not apparently, in decerebrate animals, to intermediate degrees. Of course such ratios can be expected only when there has been no stimulation, of the respirations—either hormone or nervous—before the vagi were cut. If this operation be performed during CO_2-hyperpnea, for example, then, as shown in figure 3, no simple ratio is likely between the breathing immediately before and after.

It should be added that section of the vagi sometimes causes complete breakdown of breathing in decerebrate cats, but in our experience this is the exception rather than the rule. The exact position of the cut across the brain stem is the most important factor in determining whether or not vagotomy will cause respiratory failure. In our experi-

ence, if the anterior corpora quadrigemina be intact respiratory failure does not follow vagotomy, but if the cut involve these structures to any considerable extent it is likely to occur.

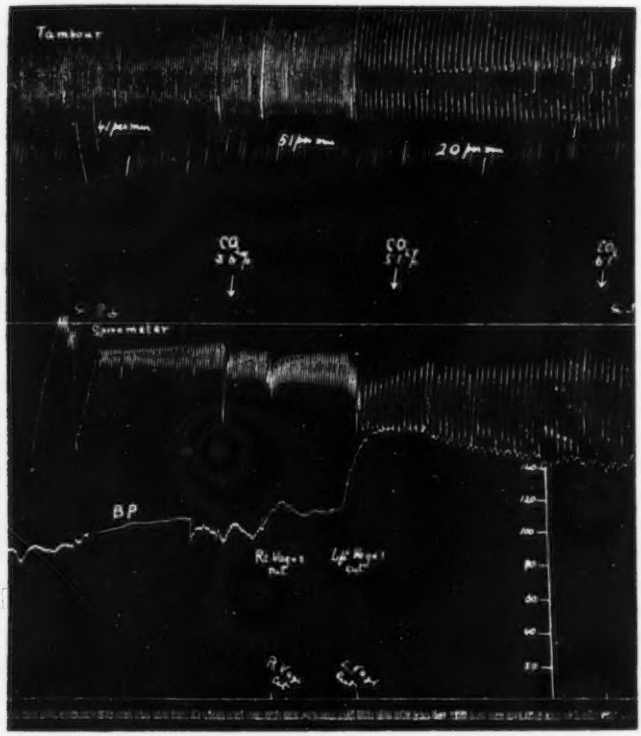

Fig. 3. (Cat 32) Cutting of both vagi in decerebrated cat caused the respirations to become slower but not irregular. At the time when the nerves were cut the animal was respiring in a closed system so that the percentage of CO_2 in the alveolar air was steadily rising, as indicated by the figures. Upper tracing, tambour; middle tracing, spirometer; lower tracing, arterial blood pressure. Time in seconds.

THE RELATIONSHIP BETWEEN THE HORMONE AND NERVOUS CONTROL OF THE RESPIRATORY CENTER. Current opinion is decidedly vague as to the precise relationship existing between hormone and afferent stimulation of the respiratory center. According to the usually accepted view

the rhythmic discharges of the center are regulated to a rate and depth that is adequate for efficient respiration, partly by the action of the respiratory hormones affecting the depth and partly by certain afferent stimuli (affecting mainly the rate) arriving at it principally by way of the vagus nerves. Afferent stimuli from other sources, and cerebral impulses, only occasionally act on the center, either exciting or inhibiting its rhythm temporarily. The question therefore arises as to whether maintained changes in either the nervous or the hormone stimulus may alter the excitability of the center toward occasional changes of the other form of stimulus.

a. The effect of vagotomy on the excitability of the center toward hormones. To determine whether the excitability of the center toward hormone stimulation becomes altered when it is almost entirely isolated from afferent stimuli, we have measured the effects produced on the minute volume of air breathed when the percentage of carbon dioxide in the inspired air is increased in decerebrate cats (by the same technique as that used by R. W. Scott (3), before and after vagotomy. The results of one of several experiments, all yielding corresponding results, are shown in the accompanying curves (fig. 4) in which the figures on the abscissa give the percentages of CO_2 in the inspired air, and those on the ordinates the number of cubic centimeters of air breathed per minute. The thick continuous line is drawn from the results obtained before vagotomy and the thick broken line from those after vagotomy in the same cat. The number of respirations is also depicted in the curves drawn in thin lines. It will be seen that there is a very close correspondence in the results of the two experiments. In no. I the initial respiratory volume is somewhat greater after decerebration than before (about 40 cc.) but by the time 2.5 per cent of carbon dioxide was being inspired the volumes have come to be the same. The rate of breathing (thin dotted line) increases along with the minute volume before vagotomy but remains unchanged after this operation until higher percentages of CO_2 are being inspired, when it may decline. During both experiments the rate of increase of breathing for different percentages of CO_2 is somewhat greater than the average given by R. W. Scott probably because a smaller volume of air was contained in the rebreathing system of tubing. This air was mixed with a large excess of oxygen, there being always well over 20 per cent of this gas present at the end of each observation. The parallelism in the curves persists until about 4 per cent of CO_2 was being breathed when the curve after vagotomy becomes less steep and at about 5 per cent actually begins to decline.

This failure of the respiratory response at higher percentages of CO_2 has not been invariably observed in other experiments of the same type.

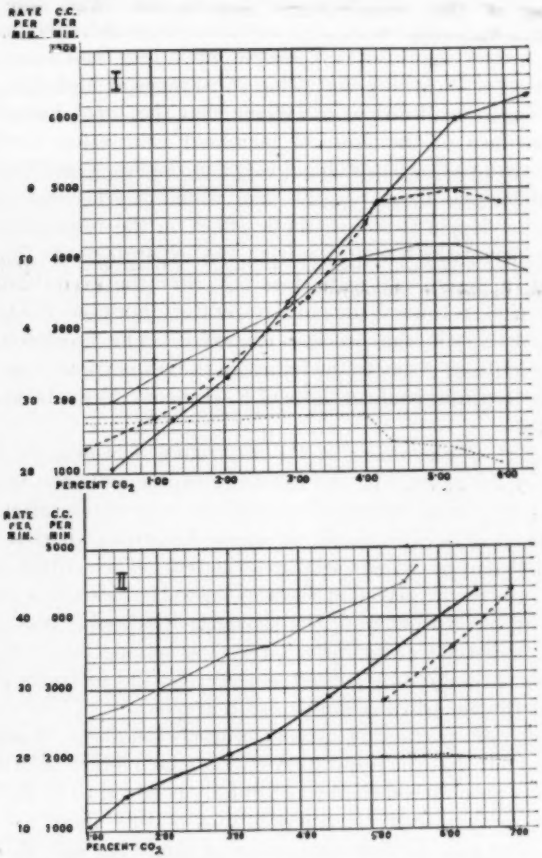

Fig. 4. Curves showing the relationship between breathing and progressively increased percentage of CO_2 in inspired air before and after vagotomy in decerebrate cats. Thick continuous and broken lines represent the minute volume of respired air, thin lines the rate of breathing per minute.

Thus it did not occur in the second series of curves shown in figure 4. When it occurs it is accompanied by a slowing of the breathing and it indicates that the limit of hyperpnea has been reached. This limit

might be determined either by the extent of expansion and collapse of the thorax or by exhaustion of the respiratory center. Examination of tracing of the respiratory movements does not throw light on this question because, being accelerated as well as deepened, each respiratory movement before vagotomy is of course much less than after it. The actual slowing of breathing that often supervenes at high CO_2 percentages after vagotomy leads us to believe that the breakdown is due in the first instance to the limit of increased ventilation having been reached so that CO_2 is less well got rid of in the lungs and by accumulating in the blood more rapidly than would be the case with freer breathing, develops a toxic anesthetic effect on the respiratory center.

The conclusion which we draw from the experiments is that section of the vagi in decerebrate cats does not alter the excitability of the respiratory center toward the chief respiratory hormone (CO_2-tension) at least until the stimulus becomes excessive. The breakdown under the latter conditions is due to the failure of the respirations to accelerate.

These observations confirm those of F. H. Scott (4) made on anesthetised rabbits.

b. The effect of alterations in the respiratory hormones on the reflex excitability of the center. In the following experiments the response of the respiratory center toward afferent nerve stimulation after intravenous injections of acid or alkali, or during breathing air that was rich in CO_2 or deficient in oxygen, was investigated. The central end of the sciatic or vagus nerve, placed in Sherrington's electrodes, was stimulated with the Faradic current and, the strength of stimulus just necessary to produce a distinct and constant response having been ascertained, the hormone stimulus was caused to vary, by the methods just mentioned, and the electrical stimulus again applied.

1. The effect of injections of sodium carbonate solution. It has already been pointed out that the respiratory effects which follow such injections are much less pronounced than was formerly believed to be the case (3). Only when comparatively strong solutions of this alkali are injected rapidly is there any decided diminution of breathing and R. W. Scott has shown that enough may be injected slowly to raise pH of the blood to 7.7 or 7.8 without much diminution in the minute volume of air breathed.

In figure 5 are shown the effects of stimulation of the sciatic nerve before and after the injection, during a period of 8 minutes, of 10 cc. of a 4 per cent solution of sodium carbonate in gum saline. According to Scott's results this should cause the blood to become decidedly alka-

line (3). With the secondary coil at 26 cm. before the injection the respirations were slowed, expiration being deepened, and there was a slight pressor effect on blood pressure. Immediately after the injection the results were as nearly as possible the same, being, if anything, slightly more pronounced. This experiment has been repeated several times and we could never convince ourselves that the alkaline injections

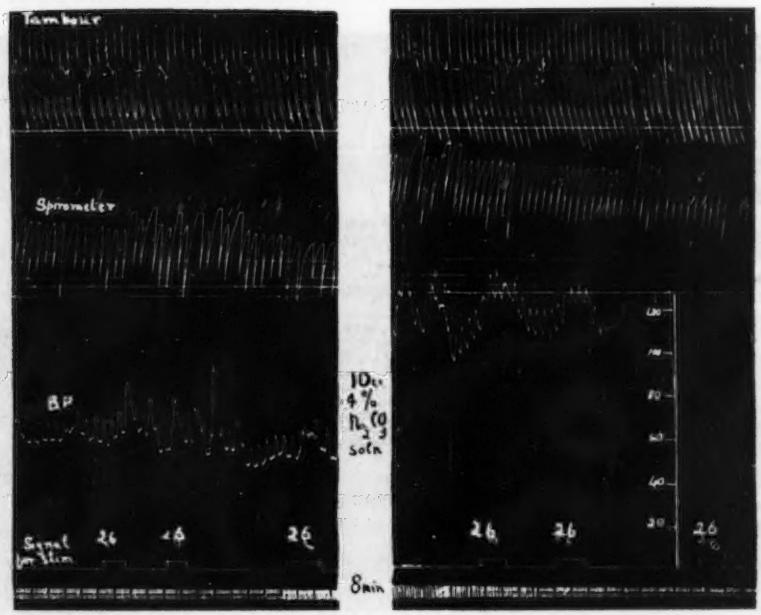

Fig. 5. (Cat 21, 2.5 kgm.) The effect of stimulation of the central end of the sciatic nerve before and after the injection of gum saline containing 4 per cent sodium carbonate. There is no evidence of depression in the excitability of the center although pH was presumably raised. Time in seconds.

had any constant effect one way or the other on the excitability of the center to nerve stimulation.

2. The effect of injections of acid solutions. These are shown in figure 6. With the secondary coil at 26 cm. inspiration was slightly excited and the blood pressure decidedly increased, and these results were not perceptibly altered immediately after the injections of as large amounts of a solution of lactic acid as the heart would tolerate. Even after

repeated injections of the acid solution, which were made in the interval clapsing between the two parts of the tracing, no increased excitability of the respiratory center could be demonstrated as is evident from the later part of the tracing.

3. The effect of breathing atmospheres rich in carbon dioxide. The objection may be raised to the above described experiments with acid on the gound that their effects on the H-ion concentration of the blood

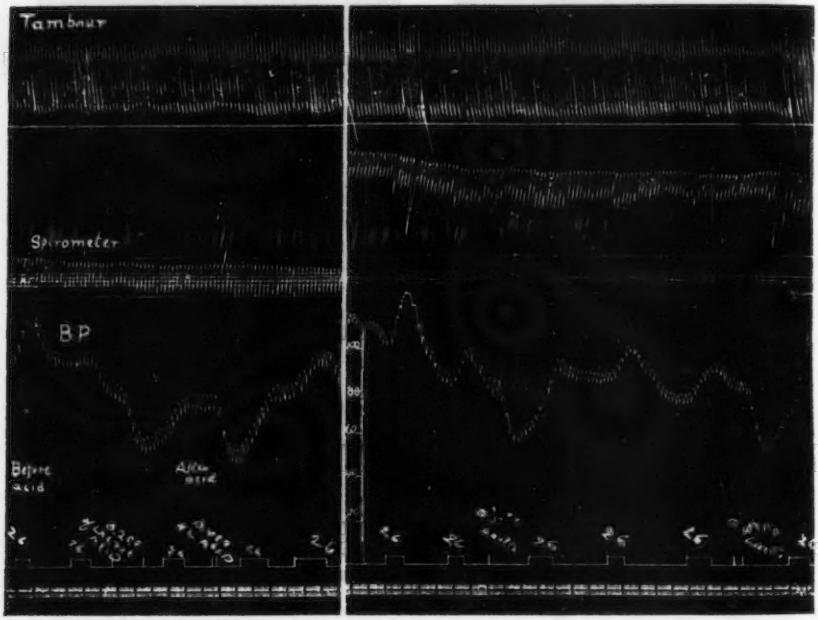

Fig. 6. (Cat 21, 2.5 kgm.) Stimulation of central end of sciatic nerve before and immediately after the injection of N lactic acid in as large amounts as the animal would tolerate. Time in seconds.

supplying the respiratory center had passed off before the second series of afferent stimuli was applied. It became necessary to supply the acid continuously, but since we have found that continuous injections of solutions of mineral acids *are very liable to cause a sudden breakdown of the respiratory mechanism, at least in decerebrate cats,* we decided to bring about the desired increase in the hormone stimulus by causing the animals to breathe atmospheres containing sufficiently high percent-

ages of CO_2 definitely to excite the respirations. The effect of afferent nerve stimulation was then observed immediately before and during the hyperpnea.

The results of such an experiment are shown in figure 7. In this case the middle tracing (spirometer) was taken with a clamp applied to the outlet tube of the spirometer with a pressure which was sufficient to make the rate of filling and of emptying just balance for ordinary breathing. The very slightest increase or decrease in the minute volume is therefore indicated by a rise or fall in the level of the spirometer tracing. It will be observed that stimulation of the central end of one vagus nerve with the secondary coil at 45 cm. caused a slight increase in the minute volume which was practically the same during a prolonged state of hyperpnea produced by respiring CO_2-rich air as it was in the preceding and following periods. During one of the periods of stimulation (marked * on tracing) the administration of CO_2 caused a slightly greater response than the usual but this is the only instance in a considerable number of experiments in which such an effect has been observed.

Similar conclusions can be drawn from the tracing shown in figure 8 in the experiment of which the outlet tube of the spirometer was completely clamped at intervals so that its rate of filling might be determined. Unfortunately the capacity of the spirometer between the two lines is not exactly known for this experiment but this does not really matter for purposes of comparison, the rate of filling being proportional to the distances between the vertical lines. With the coil at 40 cm. stimulation of the central end of the sciatic nerve caused a marked rise in blood pressure and changed the rate of filling of the spirometer on an average from 17 seconds to 10 seconds and later from 15 seconds to 12 seconds, while the animal was breathing outside air. During the hyperpnea caused by breathing a CO_2-rich atmosphere the same strength of stimulus produced less effect on the blood pressure, the rate of filling of the spirometer being changed on an average from 14 seconds to 11 seconds and later, from 15 seconds to 12 seconds. There is in these results no evidence that the excitability of the center has been altered by carbon dioxide.

Although we have been unable to confirm Cohen (5), who performed similar experiments, we are prepared to admit that the problem demands still further investigation. Inasmuch as it has been shown, especially by Collip (6), that respiratory excitement may result from intravenous injection of bicarbonate solutions and in light of the observations of Jacobs (7) on the cause for the greater exciting effect

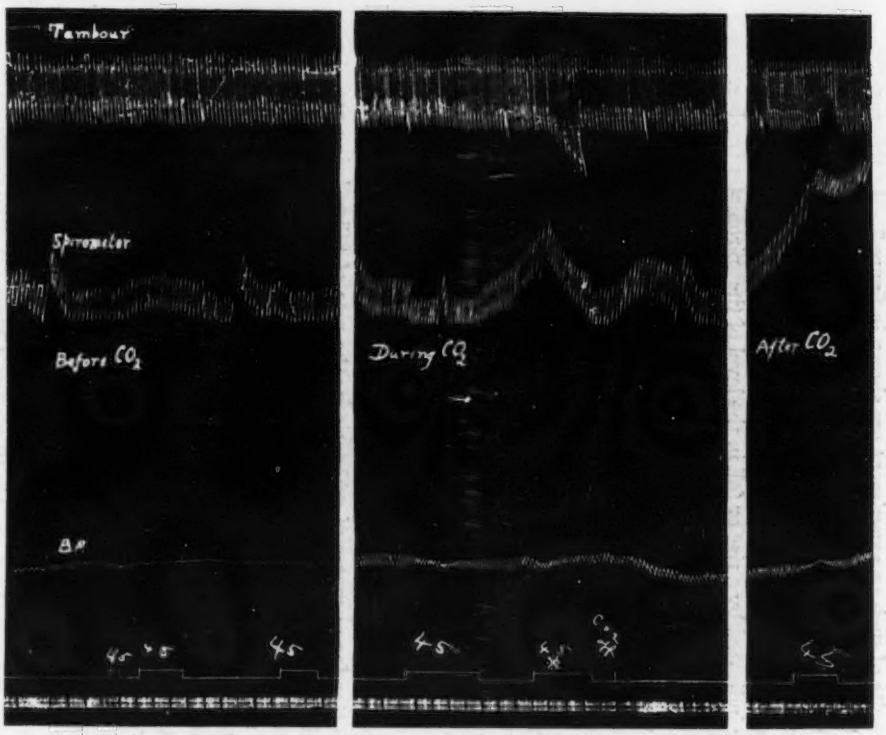

Fig. 7. (Cat 14) Stimulation of the central end of the vagus (at the periods indicated by signals) during and some time after breathing into an atmosphere containing an excess of CO_2. The respiratory tracing in this experiment was taken by connecting one tube of a Krogh spirometer with the expiration valve and partly clamping the other tube. A rise or fall in the level of the tracing, therefore, indicates increase or decrease in the volume of air breathed. The distance of secondary coil indicated by figures. Time in seconds.

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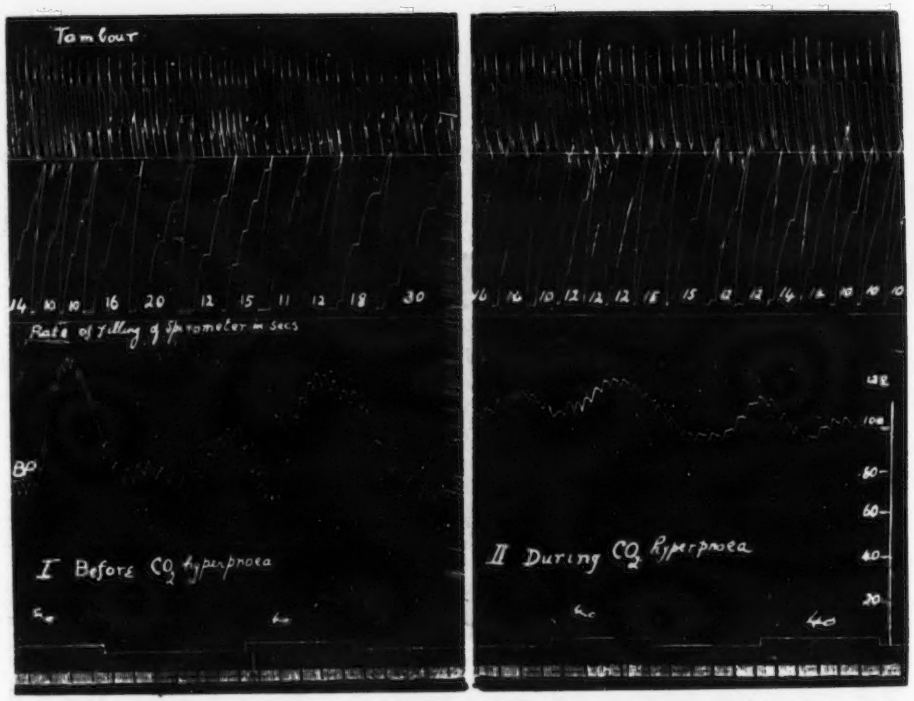

Fig. 8. (Cat 15) The effect of respiring CO_2-rich air on the excitability of the respiratory and vaso constrictor centers toward stimulation of an afferent nerve (sciatic). The uppermost tracing is that of a tambour connected with the trachea, the middle tracing shows the rate at which a Krogh spirometer filled to 240 cc., and the lowermost tracing is arterial blood pressure. The periods of stimulation are indicated by signals, the figures above which give the distance of the secondary from the primary coil. Tracing 1, breathing normal air; tracing 2, breathing CO_2-rich air. Time in seconds.

of CO_2 tension than would correspond to its effect on the CH of the blood, we propose to repeat this part of the investigation under more strictly controlled conditions of afferent nerve stimulation.

4. The effect of anoxemia. From the tracing shown in figure 9 it will be seen that the effect of stimulation of the central end of the vagus nerve

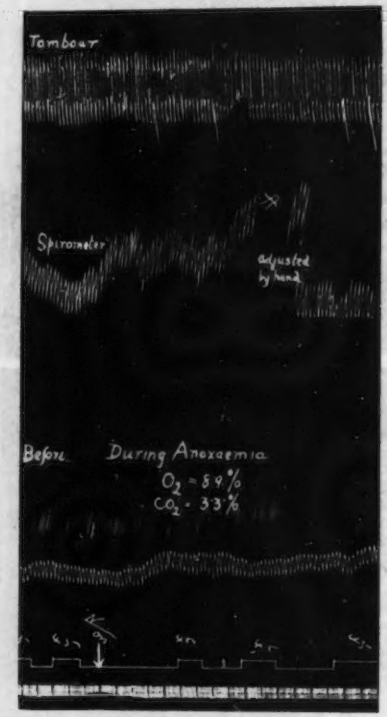

Fig. 9. (Cat 14) Stimulation of central end of vagus before and during breathing into an atmosphere very deficient in O_2. Time in seconds.

was the same when the animal was breathing in an atmosphere containing about 10 per cent O_2 as in outside air. The spirometer tracing was taken by the same method as in experiment 7. This observation has been repeated at various stages of anoxemia and always with the same result, i.e., at no stage in anoxemia can it be shown that the respiratory center is hyperexcitable to afferent nerve stimulation.

THE INFLUENCE OF SECTION OF THE VAGUS NERVE ON THE GRADUAL INCREASE IN BREATHING WHICH SUPERVENES SHORTLY AFTER CAUSING AN ANIMAL TO BREATHE INTO A CLOSED SYSTEM OF TUBES. The occurrence of this form of hyperpnea has been noted in a previous communication (4) where also it is shown that deficiency of oxygen cannot be its cause. The slight resistance that is offered to the movement of air due to friction in the tubing, by raising the intrapulmonary pressure, was considered to be the cause of the hyperpnea although a similar increase of breathing could not be induced by weighting the spirometer. The only other possible explanation of the phenomenon is that the tension of CO_2 in the alveolar air becomes raised by breathing in the closed system (even although this contains CO_2 absorbers) and, consequently, the CO_2 tension in the blood. The more or less gradual onset of the hyperpnea would lend support to this view. As a matter of fact, however, the percentage of CO_2 in the alveolar air is decidedly decreased during the hyperpnea and R.Q. is raised (cf. 4). Nor can prolongation of the dead space be held accountable for the phenomenon.

If reflex stimulation of the respiratory center be the cause, it is presumably through the vagus nerves that the afferent impulses would pass. It is therefore of interest to observe the influence of section of the vagus nerves on the phenomenon. The results of such an observation are shown in figure 2. Before section of the nerves both the rate and the depth of breathing increased gradually after connecting the trachea with the closed system whereas after the section only the depth increased. In two minutes the minute volume of respired air increased from 585 cc. to 1420 cc. with intact nerves and from 375 cc. to 750 cc. when these were cut. This result shows that a reflex through the vagus nerves cannot be responsible and we are driven to conclude either that afferent pathways from the respiratory muscles must be concerned or that some at present inexplicable change in the hormone stimulus is the cause of the hyperpnea.

CONCLUSIONS

After section of the vagus nerve in decerebrate cats, the breathing usually declines somewhat in minute volume and the rate diminishes, often in some simple ratio to the normal rate. Similar section in decerebrate rabbits causes complete breakdown of the respiratory function. Increasing the percentage of carbon dioxide in the inspired air has almost exactly the same stimulating effect on respirations before and after section of the vagi, the only difference being that with high

of CO_2 tension than would correspond to its effect on the CH of the blood, we propose to repeat this part of the investigation under more strictly controlled conditions of afferent nerve stimulation.

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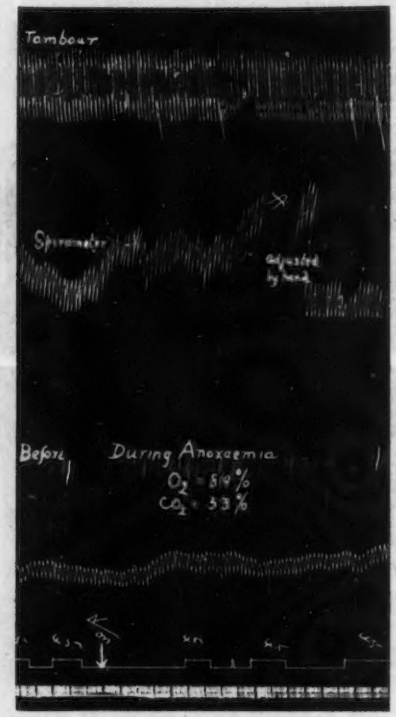

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percentages of CO_2, the respirations after section of the vagi become slower and the minute volume ceases to increase and may decline.

The excitability of the respiratory center to afferent nerve stimulation (sciatic and vagus) is not definitely increased after the intravenous injection of fixed acid or during the hyperpnea induced by respiring atmospheres rich in carbon dioxide or atmospheres poor in oxygen.

Conversely it is not decreased after the injection of sufficient amounts of sodium carbonate to lower the H-ion concentration of the blood.

These results show that the reflex excitability of the respiratory center is not altered by changes in the respiratory hormone (CH and CO_2-tension) of the arterial blood.

The gradual increase in breathing, which occurs immediately after connecting the trachea with a closed system of wide bore tubes, still occurs after section of the vagi.

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SENSORY STIMULATION BY SATURATED MONOHYDRIC ALCOHOLS

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The following investigation deals with certain effects of alcohols and particularly with the question whether in a series of isomeres branching of the chain determines the relative efficiency of the members of the series.

The experiments were performed on *Allolobophora fœtida*. Each worm was stimulated by the method described in a previous paper (1). In the present investigation one worm was used for each experiment, as suggested by Crozier (2), instead of using the same worm for a series of experiments. The worm was placed on a table surrounded by a test solution, and allowed to crawl freely to the edge of the table and enter the solution. The reaction time (which is represented by the time elapsing from the moment the prostomium of the worm enters the solution until it is withdrawn) was recorded by a stop watch. The efficiency of different members of the alcohols in the series was found by determining what concentrations bring about approximately the same reaction time. Fine distinctions are not possible, because the variation in the reaction time at one concentration is too great; even when one hundred readings are made, it is difficult to determine reaction time with greater accuracy than that obtained in these experiments.

Methyl alcohol, ethyl alcohol, n. butyl alcohol, n. amyl alcohol, iso-amyl alcohol and tertiary amyl alcohol were used. As shown in figure 1, curves *M* and *E*, and figure 2, curves *I*, *B*, *T* and *A*, the efficiency of alcohols at the given concentrations is as follows: methyl < ethyl < tertiary amyl < n.butyl < iso-amyl < n.amyl.

The same order is obtained when the criterion of the efficiency of the alcohol is its ability to bring about cessation of muscular activity, as shown in table 1.

The above experiments suggest that the nature of the action of alcohols on the sensory mechanism may be similar whether it results in

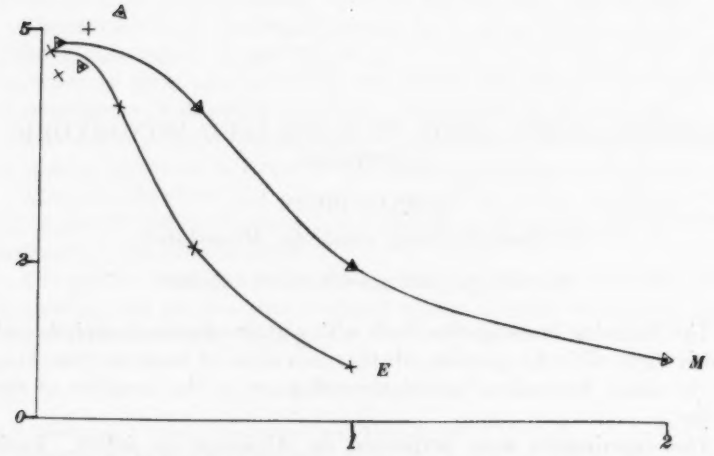

Fig. 1. Efficiency of methyl alcohol (curve *M*) and ethyl alcohol (curve *E*) as stimuli for worms. The reaction times in seconds are plotted as ordinates, and the molar concentrations as abscissae.

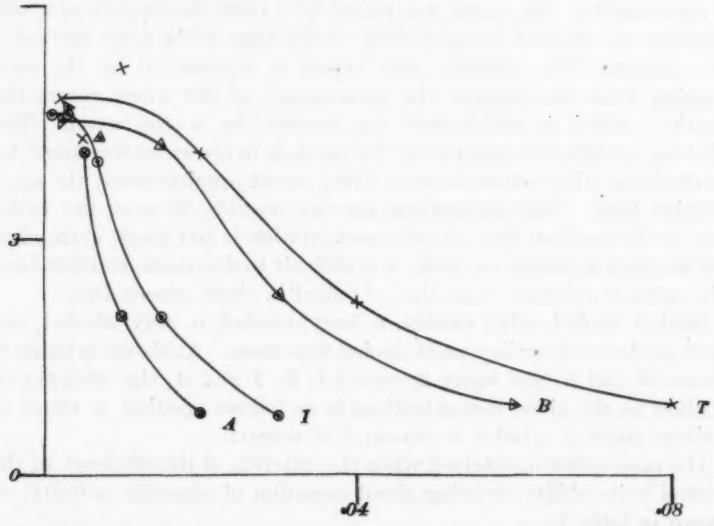

Fig. 2. Efficiency of normal butyl alcohol (curve *B*), normal amyl alcohol (curve *A*), iso-amyl alcohol (curve *I*) and tertiary amyl alcohol (curve *T*) as stimuli for worms. The reaction times in seconds are plotted as the ordinates, and the molar concentrations as abscissae.

stimulation or narcosis (3). If a certain amount of alcohol combines with a constituent of the protoplasm to produce stimulation, the combination of a larger amount may produce narcosis.

One reason why the concentration below which there is no narcosis is higher than that for stimulation is because the sensitivity of the worm decreases progressively from the anterior to the posterior portion. Therefore it requires a higher concentration of the alcohol to bring about an effect on the total body of the worm, than in the case of the prostomium, which is the most sensitive portion.

Some investigators are inclined to attribute the relative efficiency of these alcohols to their lipoid solubility (4) or to their surface tension relations (5). It may be of interest to suggest some other possibilities.

TABLE 1

Anesthetic action of alcohols on worms. Limiting concentrations below which there is no anesthesia in one hour at 23°C.

ALCOHOL	CONCENTRATION
Methyl	2.00 M
Ethyl	1.00 M
N. butyl	0.06 M
N. amyl	0.02 M
Iso-amyl	0.03 M
Tertiary amyl	0.08 M

One way of regarding the question is from a stereo-chemical standpoint (6). How far can the action of such a series of alcohols be interpreted as due to the stereo-chemical structure?

The decrease of reactivity might be interpreted as due to a steric hindrance, brought about by the arrangement of the carbon atoms in the molecule.

This idea of steric hindrance has been disputed by Michael (7), who believes that the reactivity of alcohol is independent of stereo-chemical structure, and may be more profitably interpreted from the point of view of the amount of chemical energy present.

In esterification of these alcohols, the rate of reaction decreases from amyl alcohol to methyl alcohol progressively; this is exactly the opposite of the order of efficiency obtained in the experiments described above. But when any one alcohol and its isomeres are considered, it is evident that the effect on stimulation agrees with the rate of esterification, in that the branching of the chain decreases the reactivity. Tertiary

amyl alcohol is less reactive than iso-amyl alcohol, which is again less reactive than the normal amyl alcohol. Whether the chemical effect of the alcohol is masked by a physical effect (such as solubility or surface tension relations) in the case of the normal alcohols, can not be decided at present.

It may also be suggested that the solubility of the alcohols in water may be of importance. With the branching of the chain, solubility of the alcohol in water decreases. With increase in the number of carbon atoms the solubility in water tends to decrease.[1]

Solubility in water might conceivably interfere with stimulation in a number of ways, but in view of the present state of our knowledge of this subject it does not seem profitable to enter into a detailed discussion of these possibilities.

SUMMARY

The effects of a series of monohydric alcohols on the sensory mechanism of *Allolobophora fœtida* are compared. The branching of the chain decreases the efficiency.

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[1] Data regarding the solubilities of the higher alcohols in water are for the most part very unsatisfactory. N.amyl is less soluble than n. butyl, which is, in turn, less soluble than ethyl and methyl.

STUDIES ON THE RESPONSES OF THE CIRCULATION TO LOW OXYGEN TENSION

VI. The Cause of the Changes Observed in the Heart during Extreme Anoxemia[1]

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In our previous papers we have presented the changes that occur in the human during extreme anoxemia with especial reference to cardiac physiology. In our rebreather method the carbon dioxide is absorbed by shell caustic potash, hence there is no increase in carbon dioxide of the inclosed air during the augmentation of respiratory volume. In fact there may be a decrease in carbon dioxide due to the over-ventilation of the lungs with the consequent lowering of carbon dioxide tension in the body. Therefore, the gradual reduction of oxygen in the air breathed, hence in the lungs and body tissues, may be considered as the primary cause of the physiological changes observed. The condition is a true anoxemia. We have already reviewed at some length the literature of this subject especially that developed in connection with the work of the Medical Research Laboratory of the Air Service (1) where we first began the investigation of this problem on man.

In preceding papers of this series (2) we described the late cardiac effects of anoxemia when the condition is pushed beyond the ordinary limits of the technical air service examination. What we have described as the post-crisis stage includes the changes observed in the circulatory system and especially in the heart after unconsciousness has occurred. These changes in the heart of man are summarized as follows:

[1] A grant in aid of this research was made from the Patton Research Fund of the Northwestern University for which appreciation is expressed.

We also express our obligations to Prof. Frank G. Becht for the many courtesies extended by the Department of Physiology, of the Northwestern University School of Medicine, and for generous personal assistance.

1. Progressive suppression of the S-A rhythm in the descending direction to the vanishing point.

2. Establishment and persistence of the A-V rhythm with its characteristic slow and regular rate.

3. Decrease in conduction in the internodal region to the point of suppression.

4. Reversed rhythm and reversed internodal conduction.

5. Auricular pause after reversed conduction disappears.

6. These changes obey the laws of cardiac nervous control of rhythm and conduction, as outlined by Eyster and Meek.

7. Rapid recovery from all these disturbances (i.e., within a few seconds) when the man is allowed to breathe fresh air.

Most of these phenomena had been described in laboratory animals under the condition of systemic asphyxiation, but not in man. Eyster and Meek (3) especially have advocated the view that the phenomena are primarily due to vagospasm. Others, especially those working in Lewis' laboratory, have emphasized the fact that the asphyxial stress on the heart also acts directly on cardiac tissue. In all the earlier experiments on animals two factors are involved, oxygen want and carbon dioxide excess. Mathison (4) alone has tried to separate these factors by allowing his animals to breathe pure nitrogen, thus getting rid of the carbon dioxide by excessive ventilation. He concludes that "want of oxygen . . . . . alone is responsible for the production of block." He says also: "the heart block is undoubtedly due to the effect of want of oxygen on the cardiac tissues." Mathison describes stimulation of the cardio-inhibitory center, especially after chloroform, but he closes his article with the sentence: "Heart-block appears to be a regular occurrence during asphyxia in dogs in which the vagi are cut. When the vagi are intact, permanent cardiac inhibition frequently comes on before heart-block can appear."

These quotations summarize the most direct evidence bearing on our problem that we have been able to find. There is nothing in the literature that deals directly with man which furnishes an experimental basis for analyzing the mechanism involved in the observations which we have reported.

Lutz and Schneider (5) studied the responses of men breathing pure nitrogen from a Larsen spirometer supplied from a gas bag and exhaling into outside air. By this method they produced acute anoxemia in a few seconds. They obtained cardiac acceleration in from 5 to 55 seconds, "within 15 seconds in sixty per cent of the cases." While they

obtained unconsciousness in certain tests they record no heart rates at this stage, in fact, resupplied air and studied the return phenomena before the extreme anoxemia with which we are concerned had appeared. For the purposes of our experiments such methods are too acute and do not allow full adjustment of the tissues to the external conditions. The same questions hold with this work as with that of Mathison on dogs.

We have not tried extreme anoxemia during atropinization, in fact we have had some hesitation in using this, the only method available for answering the question whether anoxemia in man produces the observed cardiac changes by direct action on the heart and its intrinsic mechanisms, or by changes in the extrinsic nervous apparatus, i.e., primarily by vagospasm. We have interpreted our human results as due to the latter phenomenon. But we have not deemed it advisable to perform the crucial tests on man without further experimental data from lower animals.

In the present paper dogs are used as experimental materials. A further attempt is made to determine observational facts on which to base an answer to the questions stated above.

Method. Dogs have been used exclusively during this series of experiments. Anoxemia has been induced after chloretone anesthesia either alone or combined with ether. We have used the rebreather method, constructing an apparatus of small size suited to animals of about 7 to 10 kilos body weight. Changes in the general blood pressure have been measured by a mercury manometer, taking the pressure from the carotid artery. Respiratory rate was recorded by the movements of the spirometer, which also recorded, though with a low percentage of accuracy, the relative tidal volume. This apparatus records the progressive changes in volume of the inclosed air, thus giving a measure of the variations in rate and amount of oxygen consumed.

Electrocardiograms were taken at intervals of about 4 minutes beginning with the normal. A continuous electrocardiogram was taken from a late moment in the pre-crisis stage through the entire post-crisis stage and till the ending of the experiment by death of the animal, or by recovery following artificial respiration.

An analysis of the enclosed air in the rebreather chamber was made at the close of the experiment. The Haldane apparatus and method were used. The sample of air analyzed was taken from the inhalent tube, in the attempt to measure the composition of the air used by the animal at the last inhalation before respiratory failure. This doubtless

gives a somewhat higher figure than the average gas content of the entire rebreather apparatus. It is recognized that the alveolar air oxygen tension is slightly lower than that found by analysis of the air from the intake tube.

Chloretone anesthesia was used to render the animal immobile. Three-tenths of a gram of chloretone in oil per kilo of body weight was injected into the abdominal cavity. Animals vary slightly in their sensitiveness to chloretone. Occasionally a second small injection was required to produce sufficiently deep anesthesia. The 0.3 gram-per-kilo dose, however, produces complete unconsciousness in about 10 minutes. Chloretone in excessive doses somewhat depresses the medullary centers, but the reactions of the animal to anoxemia are qualitatively normal. We have used ether anesthesia, also chloretone-ether, but we are confident that the use of chloretone alone is without error.

EXPERIMENTAL DATA

Progressive anoxemia has been used on twenty-one animals, a total of forty-one expermental tests, to determine the course of physiological events. After anoxemia is pushed to the stage of suppression of the respiratory movements there still remains a considerable interval during which artificial respiration quickly revives the animal. Revival permits several tests on the same dog. Since our primary purpose has been to determine the mechanism of the effects of anoxemia expressed by changes in the heart, we have made the tests in four groups: *1*, with the vagi intact; *2*, with both vagi cut; *3*, after atropine; and *4*, with the vagi cut at the moment when advanced responses are in progress in the heart.

Anoxemia with the vagi intact. The dog under chloretone and with intact vagi gives a cycle of changes in response to progressive anoxemia that is characteristic and qualitatively constant. In all essential respects the physiological changes observed are very similar to those observed in man up to the stage when, in man, the tests are terminated. In the experiments recorded here the tests were carried to a much greater extreme. The changes observed in 16 experiments on intact animals which we stress are first, in the respiratory rate and tidal volume; second, the rate of oxygen consumption; third, the blood pressure; fourth, the heart rate and sequence.

The respiratory rate and tidal volume. The changes in respiratory rate and volume during general asphyxia in man and animals have been

presented in an extensive literature. The more recent papers that present aspects of anoxemial asphyxia are those from the experiments of Hough (6), Mathison (4), Gasser and Loevenhart (7), the reports of Lutz, Gregg and Schneider (8), Ellis (9), Greene, (10) from the Air Service data, and Haggard (11). The literature is more fully reviewed in these latter papers.

The experimental data concerning the respiratory responses of the dog to progressively induced low oxygen are in manuscript in a paper by the senior author (12), but we abstract by permission from the summary of that paper:

> Chloretonized dogs during the rebreather test show the following panoramic changes in respiratory rate and amplitude. The sequence is more sure when the cycle is completed in from 15 to 18 minutes.
>
> 1. There is little change in either the rate or amplitude of respirations in the early part of the anoxemial test, i.e., in the first 50 to 60 per cent of the duration of the test.
> 2. The amplitude and tidal volume steadily increase during the last half of the test and until the respiratory crisis is reached.
> 3. The respiratory rate also increases but becomes more and more irregular as the crisis is approached.
> 4. The rate and amplitude both rapidly decrease during the post-crisis until within a few seconds all movement ceases.
> 5. The rate of oxygen consumption was remarkably uniform to the approach of the crisis when it progressively decreased until all respirations ceased.

The dog endures a surprisingly low oxygen, as a glance at our tables will show. The average of all the acceptable tests is 3.26 per cent. The extremes vary from 4.9 to 1.6 per cent. The highest content of oxygen of air that supported respiratory movements in the dog is well below the limits that produce respiratory stress and failure in the few extreme cases we obtained with men. Only one man of our series, Lt. S. A. (13), certainly reached the limit of respiratory pause. His residual oxygen in the rebreather was 7.1 per cent. Others, in light of our more recent experiments on dogs, were undoubtedly just short of the point of respiratory failure when removed from the test. The evidence is found in the cardiographic records.

The rate of oxygen consumption, which of course varies with the size of the animal according to the surface area, is very uniform up to the moment of the onset of the respiratory crisis. From this point until the respirations cease the rate of oxygen consumption progressively diminishes (see exper. 38 and 41, figs. 24 and 34). Following the last respiration the base line usually falls somewhat in the record (see exper.

TABLE 1

Showing the entire group of experiments of the series. Chloretone anesthesia was produced by injecting a saturated warmed solution in oil into the peritoneal cavity. To the volume of air recorded for the rebreather must be added the volume of the dead space of the apparatus, about 1100 cc., and the air of the respiratory passages. Carbon dioxide was not always perfectly absorbed in the earlier experiments.

DATE 1920	EXPERIMENT	DOG	WEIGHT	CHLORETONE PER KILO	RESPIRATIONS STOPPED	AIR AT BEGINNING	AIR AT END	OXYGEN REACHED	CO_2	VAGI CUT OR INTACT	ELECTROCARDIOGRAMS
			kgm.	*grams*		*liters*	*liters*	*per cent*	*per cent*		
5-20	1	1		None		6.0				Intact	No
5-20	2	1		None	15′ 00″	6.0	3.37*	2.44		Intact	No
5-21	3	1		None	10′ 00″	5.0		4.4		Intact	No
5-21	4	2		None		5.0		6.1		Intact	No
5-22	5	1		None	14′ 00″	6.0	3.75*	2.7	0.02	Intact	No
5-24	6	3	11.4	0.3	17′ 15″	6.0	4.5	3.83		Intact	No
5-24	7	3	11.4	0.3	14′ 30″	5.0	3.9			Intact	No
5-24	8	3	11.4	0.3	17′ 00″	5.0	3.87	3.62	0.45	Cut	No
5-25	9	5		0.3	21′ 50″	5.0	3.9	3.82	0.10	Intact	No
5-25	10	5		0.3	19′ 20″	4.0	3.12	3.71	1.65	Cut	No
5-25	11	5		0.3	19′ 45″	3.0	2.37	3.11	2.66	Cut	No
5-26	12	6	20.0	0.3	11′ 12″	6.0	4.8	4.09	4.00	Intact	Yes
5-27	13	7	13.0	0.4	14′ 00″	4.0	3.05	2.58	0.68	Intact	Yes
5-27	14	7	13.0	0.4	5′ 00″	Short special test		1.6	0.09	Cut	No
5-27	15	7	13.0	0.4	13′ 42″	4.0		4.06	1.5	Cut	No
5-27	16	7	13.0	0.3	10′ 48″	4.0	3.15	3.05	1.48	Cut	No
5-28	17	8	11.4	0.2†	13′ 00″	4.0	3.15	2.59	1.63	Intact	Yes
5-28	18	8	11.4	0.2†	12′ 15″	3.0		2.76	0.93	Intact	Yes
5-29	19	9	9.0	0.3	15′ 20″	4.0	3.25	4.46	0.43	Intact	Yes
5-29	20	9	9.0	0.3		3.0		4.44	Trace	Intact	Yes
6-4	22	11	10.0	0.3	15′ 00″	3.0	2.2	3.27	None	Intact	Yes

6–4	23	11	10.0	0.3	10′ 15″	3.0	2.25	2.34		Cut at crisis	Yes
6–4	24	11	10.0	0.3	Short special test			1.87	None	Cut	Yes
6–5	25	12	10.0	0.3	14′ 15″	4.0	3.05	5.02	1.04	Intact	Yes
6–5	26	12	10.0	0.3	13′ 30″	3.0	2.3	5.19	0.46	Intact	Yes
6–5	27	12	10.0	0.3	15′ 20″	3.0	2.37	5.52	1.37	Cut	Yes
6–5	28	12	10.0	0.3	16′ 05″	3.0	2.3			Cut	Yes
6–10	29	13	9.0	0.3	14′ 00″	3.0	2.07	2.2	None	Intact	Yes
6–10	30	14	9.0	0.3	8′ 20″	3.0	2.2	4.27		Intact	Yes
6–10	31	14	9.0	0.3	7′ 30″	4.0	3.22	5.46		Atrop.	Yes
6–11	32	15	8.0	0.3	15′ 30″	3.0	2.2	4.17	Trace	Intact	Yes
6–12	33	16	7.5	0.3	12′ 20″	3.0	2.32	4.37	Trace	Intact	Yes
6–12	34	17	10.0	0.3†	16′ 15″	4.0	3.05	2.94	Trace	Intact	Yes
6–12	35	17	10.0	0.3	13′ 42″	3.5	2.65	2.87	None	Cut	Yes
6–14	36	18	10.0	0.3†	10′ 25″	3.5	2.42	3.46	Trace	Cut	Yes
6–14	37	19	9.0	0.3	18′ 30″	3.5	2.5	2.43		Intact	No
6–14	38	19	9.0	0.3	14′ 30″	3.0	2.2	2.38		Intact	No
6–14	39	19	9.0	0.3	Short special test			2.2		Intact	No
6–15	40	20	16.0	0.3	8′ 54″	4.0	3.25	2.5		Intact	Yes
6–15	41	21	19.0	0.3	11′ 00″	4.0	2.92	1.8	0.10	Cut	Yes

* The trial face mask used probably leaked on expiration.
† Ether.

38, fig. 23). The explanation is that when the dog stops breathing the muscles relax and the chest volume decreases, forcing some of its alveolar air into the rebreather apparatus. When the respirations cease the tracheal tube is clamped off from the rebreather and the rebreather air then analyzed. The analyses therefore represent the percentage of oxygen in inspired air that just fails of maintaining respirations.

Effects of anoxemia on blood pressure and on the heart rate. Both the blood pressure and the heart rate respond typically and with fair constancy to anoxemia. The rebreather method with the taking up of carbon dioxide by the potash cartrige absorption induces anoxemia so gradually and evenly that the conditions can be repeated with great accuracy. The experimental cycle of changes constantly recur with only very minor variations.

Blood pressure changes in the intact animal. Blood pressure remains remarkably constant for the first half or two-thirds of a rebreather test on the dog. This is shown very well in experiment 38 with the vagi intact. In this test there was little or no change in the blood pressure for the first 10 minutes of a 16-minute experiment. Beginning at about 10 minutes, the blood pressure very slowly increased through 2 or more minutes, then more and more rapidly until the maximum was reached at the time of the vascular crisis. This crisis coincided very nearly with the respiratory crisis.

Sometimes the maximal blood pressure lags a few seconds after the respiratory failure. The absolute rise in blood pressure varies in different experiments. It may amount to as much as 40 or 50 per cent of the initial pressure (see exper. 41). Occasionally the rise in pressure is only slight. In all cases it occurs at the extreme stage of anoxemia. The rise in blood pressure is accompanied by an increase in pulse pressure. This change is never so great in the dog but that the diastolic phase, or minimal pressure is as great or greater than the normal. In man the diastolic pressure seldom increases to any appreciable degree during an official rebreather test but it always falls rapidly near its close if the test reaches the limit of compensation.

Events occur very rapidly in the circulatory post-crisis. The salient events are: progressive slowing of the heart and gradual lowering of blood pressure early in the collapse followed by a rapid fall in both rate and pressure later (see exper. 41).

In the crisis and during the post-crisis stages blood pressure undergoes greater and more rapid variations. The rule is that the pressure very

gradually falls during the post-crisis and until the respirations cease. The heart slows down during this phase and the pulse amplitudes increase. Consequently the diastolic pressure falls rapidly while the systolic pressure is maintained or may even rise. The maximal systolic pressure of this cycle is usually not reached until 20 to 40 seconds after respirations cease. The pressure events just described are followed by progressive and rapid decrease of both systolic and diastolic pressures with decrease in pulse rates until the pulse can no longer be distinguished and the pressure remains constant at about 15 to 20 mm. Hg. The length of time required for the entire post-crisis cycle is from 3 to 5 or more minutes after respirations cease.

Simple artificial respiration or insufflation suffices quickly to resuscitate an animal during the time when the pulse is still distinguishable on the manometric record. Later than this additional measures must be employed. Recovery of both heart rate and blood pressure when they occur are prompt, i.e., within 10 or 15 seconds.

The comparison between our results and those obtained by the methods of rapid deprivation of oxygen as practised by Mathison (4) and by Lutz and Schneider (5) is rendered difficult because in such experiments the results are brought on by immediate and rapid asphyxiation, i.e., within a few seconds. Mathison produced asphyxiation in his animals by stopping artificial respiration, and by using nitrogen gas with or without a minimal amount of oxygen, 1 to 2 per cent. In either case the transition from normal air to the asphyxial condition is sharp and abrupt. Schneider and Lutz had men rebreathe nitrogen gas into a small bag. Our animals are allowed 10 to 18 minutes to gradually exhaust the oxygen from the air they rebreathe. There is adequate time for slow and gradual adaptation.

When natural respirations stop it can be assumed that the tissues are already deprived of their free oxygen. The low oxygen content of the last inhaled air is the basis of this deduction. In anoxemia there is not such an abrupt and violent response in blood pressure as was obtained by Mathison. On the other hand, the rise in blood pressure is very gradual, generally uniform, and passes away with the same progressive type of readjustment.

Blood pressure changes when the vagi are cut. If anoxemia is induced after both vagi have been cut the blood pressure runs a course qualitatively very like that in the intact animal. The variations are chiefly those conditioned by changes in heart rate and pulse pressure. The detailed picture is as follows:

The average blood pressure does not vary during the early part of the test as much as in the intact animal. But as stress from oxygen-want becomes more acute the blood pressure rises as in the normal animal. The rise progressively increases to a maximum at the anoxemial crisis. Very little difference exists in either the rate or time of development of the crisis. The type change is shown in the last part of figure 34.

After respirations cease, sometimes a little earlier, the blood pressure slowly declines through 40 to 60 seconds. It then may show a slight increase, but finally falls rapidly through 2 or 3 minutes, then more slowly for 1 or 2 minutes more until the positive pressure of 15 to 20 mm. Hg. is reached.

If both vagi are cut the anoxemial curve of the dog never shows the enormous variations of pulse pressure during the early post-crisis stages. The response is in sharp contrast with the responses when these nerves are intact. We have never obtained confirmation of the lowered but sustained blood pressure associated with the slow heart rate, large pulse amplitude and heart block as given for the cat with the vagi cut in Mathison's experiments (see his fig. 5) (4). In dogs with vagi cut the final stages of anoxemial heart block have never appeared until the pressure approached equilibrium and the heart beats were no longer recorded by the manometer.

Changes in the heart rate in the intact animal. The heart rate is very uniform during the first 50 to 60 per cent of the anoxemial test. Unless stimuli from the outside occur, this regularity is uninterrupted. Sooner or later, varying somewhat with the animal, the heart rate slowly and gradually augments. This increase continues along with the increase in blood pressure previously described. Whether the increase in rate is the chief factor producing the rising pressure has not been determined in this investigation but Mathison speaks of vasomotor stimuli in the spinal animal. In an experiment running 15 minutes the increase in rate will be very apparent by the 10th or 11th minute. It will progressively augment to a maximum at 13 or 14 minutes. The maximum rate is associated with or slightly precedes the maximum blood pressure. This group of responses of maximal heart rate, crest of blood pressure and slowing and stopping of respirations is the complex for which we use the term crisis.

After the rise of blood pressure passes its crest and while the respirations are beginning to slow and oxygen consumption is obviously decreasing, the heart rate also begins to slow. The decrease in heart rate is very gradual at first but rapidly becomes increasingly slower

until the maximum rate is cut to a half or a third. In experiment 41 the slowing was from 161 to 44 beats per minute in 70 seconds.

If an animal with intact vagi is allowed to continue in the anoxemial state then the heart rate remains slow after the blood pressure falls, often stops for a few seconds at a time, and ultimately ceases altogether. The electrocardiographic record shows that beats continue many seconds after the manometer fails to record pressure changes. It takes an average of 3 or more minutes to run this cycle of changes after respirations cease.

Changes in the heart rate when the vagi are cut at the beginning of the test. If the vagus nerves are cut before beginning the experiment, the heart rate is of course at a higher level. However, for the first 50 or 60 per cent of the duration of the experiment there is no other change in the character of the rate.

During the last third of the experiment, passing through the crisis as indicated by the maximal blood pressure and stopping of respirations, the heart rate augments in the pre-crisis period and continues at a rapid rate during the post-crisis. There is no early cardiac slowing to the extremely low rate observed when the vagus nerves are intact. The rate remains uniform and high for from 1½ to 2 minutes after respirations stop and until the blood pressure is falling rapidly. By the moment the blood pressure has declined to one-half its earlier maximal the heart rate has become very evenly and gradually slower. It beats more and more feebly until it stops or until irregularities develop. When the heart is beating too feebly to produce any visible movement of the meniscus of the manometer the electrocardiograms show it to be still contracting in a normal sequential rhythm. It keeps this up for many seconds but ultimately block or independent auricular or ventricular beats are established and death follows.

The early cardiac slowing observed in dogs with intact vagi does not occur in our animals with vagi cut. Neither is there any evidence of change in conductivity, or block in the early phase of the post-crisis period. These come only 3 to 5 minutes later and are only revealed by the electrocardiograms.

Changes in the heart rate and blood pressure as influenced by cutting the vagi at the maximum slowing of the early post-crisis period of the intact animal. The discussion of the preceding topics clearly indicates that there are two critical times as revealed by the changes in heart rate during the post-crisis period. The first is at the time of cardiac slowing in the normal intact animal at or near the moment when respirations

stop. The second is the cardiac slowing that comes 3 to 5 minutes later in an animal in which the vagi are cut at the beginning of the experiment.

If at the moment of maximum slowing of this early period the vagi are cut then the whole situation is rapidly altered. The facts are revealed by close comparison of typical experiments, i.e., Nos. 38, 40 and 41. In these experiments we secured complete respiratory, circulatory and, in 40 and 41, electrocardiographic records without interruption through the entire post-crisis period. The vagi were cut in succession when the heart rates had dropped to between 40 and 50 per minute.

In experiment 38 the rise of blood pressure at the crisis was moderate but the heart slowing came on rapidly, the rate dropping from 176 at the crisis to 76 and then to 48 per minute. The right vagus was cut first, at 15 minutes from the beginning of the test and 45 seconds after respirations stopped. There was a sudden but momentary rise in blood pressure and an increase to a heart rate of 88, figure 23.

The left vagus was cut 40 seconds after the right. The heart rate immediately increased to 172, then 216. The original maximal rate was 174. The blood pressure at once rose to the maximal systolic pressure during the preceding period of slow heart beats, then as rapidly fell through 10 seconds, and more slowly through the next 40 seconds. Artificial respiration was then established and the animal promptly recovered.

Cutting the right vagus led to an increase of rate from 48 to 88. The high pulse pressure however continued. Cutting the second or left vagus released the heart at once to its maximum rate at the crisis. One can not escape the deduction that the extreme post-crisis slowing was a vagus phenomenon, i.e., vagal spasm, from which the heart was immediately released when the vagus nerves were cut.

Experiment 41 was also used to test the effect of cutting the vagus nerve during the early slowing in the post-crisis period. The maximal blood pressure was high in this experiment, about 50 per cent above the pre-crisis average. The heart rate slowed during the interval of 45 seconds between the maximum blood pressure and the stopping of respirations. The manometer failed to record a few beats near the moment of stopping of respirations but this does not veil the fact of the rapid and progressive cardiac slowing up to the moment when the right vagus was cut 35 seconds after respirations ceased.

For five heart beats preceding the cutting of the right vagus nerve the rate was at its lowest, 35 per minute. After one or two irregular

beats at the moment of cutting the heart remained very regular and strong at the rate of 44 per minute. This was adequate to maintain the pressure at a uniform level during the interval.

The left vagus was then cut with a minimum amount of manipulation. The heart rate immediately rose from 44 to 180 per minute. The blood pressure was momentarily increased but followed by a fall at first rapid, then more slowly, until no further heart beats could be shown in the record of the manometer. The rate was well sustained until the pressure became low. Then the rate, too, slowly declined just as in experiments when the vagi were cut at the beginning.

A continuous electrocardiographic record was maintained until no heart beats were visible by this means. The details obtained by this method are given later. No effort was made to resuscitate this animal.

Summary from the blood pressure records. The blood pressure records alone seem to prove that there are two post-crisis periods of slowing of the heart rate in anoxemia, the first a function of the vagus center, vagospasm, and the second a direct effect of oxygen-want on the heart itself. The electrocardiograms complete the evidence. We may therefore summarize the observations from blood pressure records obtained by carrying anoxemia to the complete limit of stopping respirations and heart beats.

1. The reactions of the respiratory center of the medulla become at first slow, then cease. When lack of oxygen is pushed to the death there is a phase during which the respiratory center does not receive enough oxygen to maintain its normal discharges. Finally it ceases physiological activity from true anoxemia.

2. The inhibitory centers controlling heart rate do not fail as early as the respiratory mechanisms. This is indicated by the appearance of the maximal cardiac slowing after the respiratory center has ceased.

3. The cardiac slowing in the early post-crisis stage is not due to cardiac failure, i.e., muscle and bundle failure, since it does not occur if both vagus nerves are previously cut.

4. Direct cardiac anoxemia is not adequate to suppress heart activity until from 3 to 5 minutes after respiratory failure.

5. The extreme slowing occurring after respiratory failure is promptly removed only after cutting both vagi. It is immaterial which nerve is cut first in so far as the gross rates are concerned, though differences exist in the behavior of the heart controlled by the right or the left vagus only.

6. The extreme slowing is due to vago-spasm which suppresses S-A rhythm. It is not ordinarily adequate to suppress A-V rhythm until anoxemia approaches a direct fatal effect. This slow rate therefore is an A-V rhythm released by vagus inhibition of S-A rhythm under the stress of anoxemia.

7. In extreme anoxemia when the vagi are intact inhibition may suppress the A-V rhythm. But when it occurs, a rhythmic center develops in the bundle branch, as in experiment 26, figured in plate I, figures 4 and 5.

8. Considering the heart itself, it is proven that there is an interval of from 3 to 5 minutes following respiratory failure during which cardiac beats are maintained. The rate becomes progressively slower and slower. At any moment during this interval a supply of fresh oxygen by artificial respiration is adequate promptly to recover circulatory and respiratory efficiency and remove the vagal inhibition.

9. What we have proven true for the dog checks so closely with our observations on man in the early stages of post-crisis anoxemia that we can not but believe that the mechanism of the reaction is the same in man and the dog in the final stages of progressive loss of respiratory and circulatory function.

10. It follows that in man asphyxiation by anoxemia has a considerable margin of safety provided only that a few whiffs of oxygen can be introduced into the lungs within the 3-to 5-minute intervals during which the heart continues to beat following respiratory collapse. This interval is critical and success does not always follow artificial respirations in the chloretonized dog when no other aid to resuscitation is used.

EVIDENCE FROM THE ELECTROCARDIOGRAMS

The electrocardiograms presented in the plates are all taken with the lead II. The lead was from the right shoulder to the left leg. Small nickel-plated electrodes were inserted through a slit under the skin and stitched into place for the early tests, but later nickel-plated binding posts were screwed directly into the head of the right humerus and into the shaft of the left femur. This last method proved very satisfactory and most convenient.

The normal dog electrocardiograms most often obtained are illustrated in either of the three normals in plate II, figure 8, plate IV figure 25, and plate V, figure 35. The R is very tall and the T negative or at best diphasic as in figure 35.

As anoxemia proceeds the most typical change is in the T wave. It becomes positive, then increasingly taller until at times the T is as tall as the original R (figs. 30 and 41). The maximum T is usually obtained at and following that stage of anoxemia in which respirations have just ceased. Figures 41 and 42 illustrate the change in the amplitude in the R which decreases and the S and T which both augment during extreme anoxemia following sectioning of the vagi and preceding complete cardiac anoxemial asphyxiation. This cyclic increase of the T running through the post-crisis was obtained over and over again. It apparently does not depend upon change in the position of the heart. The early experiments were performed with the animal lying on its back. But later the animal was turned to a 45° angle toward its left side. In this position the filled ventricle would tend to fall toward the left at all stages of the test.

The changes in the duration of the different phases of the electrocardiograms in the main coincide with those already described for man (1). With acceleration up to the crisis there is a perceptible shortening of both P-R and R-T intervals.

Post-crisis changes in the electrocardiograms when the vagi are intact. At the onset of the anoxemial crisis the blood pressure passes its crest and the heart rate becomes gradually slower; plate II, figures 9 and 10, plate IV, figure 27, and plate V, figure 38, all show this early slowing. This stage occurs at or preceding the moment of the stopping of respirations. Progressive slowing continues until the rate drops to one-half or one-third the normal. During the slowing the T wave greatly increases without other profound change.

Often the rate suddenly shifts, as in plate V, figure 39, to a lower level during which profound change in the type of electrocardiogram occurs. In experiment 41 the change came at the sixth complex of figure 39. The five preceding complexes show progressively longer P-R intervals, showing delayed conduction, at the sixth and two succeeding complexes S-A rhythm disappears and A-V rhythm becomes established. In the sixth complex the P wave is superimposed on the positive limb of the T. In the seventh it succeeds the T. In both cases the internodal conduction is reversed, i.e., proceeds from the A-V node toward the auricle. However, conduction is sharply delayed in the seventh complex.

The same phenomenon is shown in figure 11, plate II. The shift to A-V rhythm occurred in the second complex and those succeeding as described in the protocol of this experiment, no. 29. In plate I, figure

2, A-V rhythm was established in the third complex and continued with reversed conduction through a series of 29 beats, the last of which is shown in figure 3 of this plate.

In plate III, figure 19, a type of anoxemial influence is shown, undoubtedly of vagus origin, namely, a primary influence on the conducting bundle. A 2–1 block appeared for four groups with a progressive decrease in the P-R interval, signifying a simultaneous displacement of the rhythmic center toward the tail of the S-A node. In short, the vagus here produced its strongest effect on conduction but it also inhibited rhythm to a degree. Later in the course of the anoxemia internodal conduction was occasionally blocked and rhythm of both auricles and ventricles was enormously slowed.

The type of vagus action which drives the rhythmic center toward the tail of the S-A node is best shown in figure 28, plate IV. This figure illustrates one of a series of groups of such variations in which there was a periodic return of the normal P-R interval (see protocol, exper. 40).

The most extreme type of inhibitory displacement of rhythm is illustrated in figures 3 and 4 of plate I. After 49 consecutive beats arising in the A-V node the rhythmic focus suddenly shifts to a center in the left bundle branch, the second complex in figure 3. This type continued for 10 complexes at a rate of 26 per minute. Recovery occurred promptly on admitting air, the last complex in figure 4. The last complex in figure 13, plate III, experiment 29, also shows a rhythm proceeding from a center in the left bundle branch. In neither of these two unique cases did conduction reach the auricle.

No reference has been found in the literature to any instance of a rhythmic center so low in the bundle system. But Dr. Frank N. Wilson has very kindly sent us a very clear electrocardiogram showing displacement of the pacemaker of this class which he obtained in the dog quite incidental to other experiments.[2] "The animals were given large doses of morphine and this sometimes produced marked inhibition. In this particular animal a center located in the left bundle branch escaped and transitional complexes of the type mentioned by you occurred. In this instance I think no alternative explanation is possible." By Doctor Wilson's permission, this additional evidence is presented in figure 43, plate VI. Eyster and Meek's conception that the vagus suppresses cardiac function in the descending direction is carried a step farther by these two experiments than has previously been suspected.

[2] Private communication. Quoted by permission.

Electrocardiograms when the vagi are cut. If the vagi are both cut at the beginning of an anoxemial test, then no irregularities of the electrocardiographic complex occur in the early post-crisis period. It has already been explained that under these circumstances the rate is not slowed until late in the post-crisis asphyxiation, from 3 to 5 minutes or longer. Although the heart rate ultimately becomes gradually slower and finally stops, or becomes irregular, there is a long series of perfectly normal complexes, a series that extends through the slow and irregular rates of the early post-crisis shown in figures 1, 7, 18, 23, 24 and 34, and in the electrocardiograms of the corresponding stages. Release from these early irregularities is best shown in plates IV and V illustrating the effects of cutting the vagus nerves in succession during the early crisis.

Electrocardiographic changes when the vagi are cut during the early crisis. In plate IV, figures 29 and 30, the vagi were cut in succession at the moments indicated. When the first nerve was cut, in this case the left, there was little immediate effect on the rhythm but the P wave was changed to a negative. A-V rhythm was permanently established and conduction was apparently reversed but with occasional reversed block. Incidentally this illustrates the dominant influence of the right vagus on the rhythmic mechanism in contrast with the effect of cutting the left vagus first as shown in plate V, figures 39, 40 and 41. When the right vagus was cut first, the immediate effect was a release to S-A rhythm. However, block was established at first in the 2–1 ratio and later complete, as shown by the independent S-A and A-V rhythms persisting until the second nerve was cut, figures 39 to 41. These two experiments illustrate observations made by Cohn (13) showing the preponderance of influence of the right vagus on rhythm and the left vagus on conduction, except in our case the fact is brought out by anoxemial stimulation of the vagal center.

When the second nerve was cut in experiments of this type there was always an immediate escape to a rapid rhythm and a perfectly sequential beat that persists through several minutes, 3 to 8, before abnormality of the electrocardiographic complex was again displayed. Figures 30, plate IV, and 41, plate V, illustrate such escape.

Figures 6, plate I, 31 of plate IV, and 42 of plate V, illustrate the effects of direct cardiac final anoxemial asphyxiation. These figures present terminal stages of the series of complexes after the vagi are both cut. They are always in the late or terminal post-crisis stage of anoxemia. The first two figures show a final block of conduction with

persistence of S-A rhythm. Anoxemia here reduces the conductivity of the bundle system at a time when rhythmicity is still persistent in the upper node. The A-V node is also reduced in rhythmicity though that property is not always completely suppressed. In both experiments after a time occasional independent ventricular complexes occur. These are illustrated in figures 32 and 33.

The tracing in figure 42 illustrates the terminal anoxemia in which rhythm was first suppressed. Whether conduction was still possible could not be determined since all rhythm was suppressed.

This series of electrocardiograms on anoxemial dogs confirms our suspicion that the slowing of rate and suppression of sino-auricular rhythm in man in the early post-crisis stage is a vagus effect. This stage is entirely removed in the dog when the vagi are sectioned. Freed from vagus influence, the heart is capable of sustaining an effective rhythm for some seconds and a physiological rhythm detectable by the electrocardiograph for at least 3 to 8 minutes. The series clarifies the entire group of questions as to the relative danger in procedures which induce human anoxemial asphyxiation.

Summary of electrocardiographic changes in the dog during the post-crisis stages of anoxemia: 1. Electrocardiograms reveal the fact that the early post-crisis cardiac slowing is a strictly vagal influence on rate.

2. The degree of vagal anoxemial stimulation may completely inhibit the S-A rhythm or only drive the rhythm to a lower focus in the tail of the node.

3. When S-A rhythm is inhibited A-V rhythm becomes dominant but at a lower rate plane, 40 to 50. When A-V rhythm is established internodal conduction may still persist but in the reversed direction, producing an inverted sequence.

4. Extreme anoxemial inhibition drives the rhythmic center down into the bundle branch, in the demonstrations described in this paper the left bundle branch. Rhythm may persist here with fairly regular sequence through a demonstrated series of 10 beats. Rhythm may be entirely suppressed.

5. When the first vagus nerve is cut during anoxemial vagal stimulation the type of electrocardiogram changes, according to which nerve is cut first. If the right is cut first then the S-A rhythm often reappears but interference with conduction persists so as to produce inhibitory block. If the left is cut first then A-V rhythm persists with reversed conduction or reversed block.

6. When the second vagus is cut the heart always leaps forward to

a rapid rhythm with even greater acceleration than during the pre-crisis stage. The electrocardiograms show that this rhythm is perfectly normal and sequential in type.

7. After a prolonged series of vagus free beats, through several minutes in experiment 40, through 400 consecutive beats in experiment 41, direct cardiac anoxemia occurs. Direct anoxemia slows the S-A rhythm as shown in all experiments, suppresses internodal conduction first as in experiments 36 and 40, or suppresses rhythm first as in experiment 41. At this stage of anoxemia the A-V center does not take on the rhythm but may occasionally discharge beats. The S-A center however is apparently last to become quiescent under direct cardiac oxygen want.

GENERAL DISCUSSION OF THE RESULTS

Early papers by Sherrington (14), Roaf and Sherrington (15), Lewis and Mathison (16), and Mathison (4) present the initial literature describing heart block as a result of asphyxia in the mammal. These authors used decerebrate, atorpinized and uninjured cats. A careful reading of their papers clearly pictures heart block as an interruption of auriculo-ventricular conduction associated with a great slowing in the heart rate. Lewis and Mathison describe prolongation of the P-R interval as introductory to simple heart block beginning with a 2-1 rhythm and leading up to complete block. They describe complete dissociation, also "a marked retardation of the auricular rate and this likewise is independent of inhibitory influences," with speedy and complete recovery. Clearly they exclude the phenomenon of inhibition. Mathison attributes heart block to "lack of oxygen rather than accumulation of carbon dioxide." He says "cardiac inhibition frequently comes on before heart block can appear," but obviously he does not associate heart block and inhibition as causal phenomena. He reports heart block in dogs when the vagi are cut.

We are unable to confirm heart block at the stage described by the above authors as a change initiated locally in the conducting tissues. Without exception our experiments on dogs have never shown the pronounced early slowing with heart block if the vagi are first cut. The initial heart block is present if the vagi are intact, absent if the vagi are cut in dogs. We agree with Mathison that the phenomenon is strictly due to a lack of oxygen. But the lack of oxygen leads to a stimulation, then suppression of respirations and to a profound increase in activity of the vagal center either overlapping or quickly following the stage

at which respirations cease. If the vagi are not injured and anoxemia is allowed to take its course without artificial respiration, there is always a composite picture ultimately showing depression of conduction to the point of block; slowing of the auricle, as we think, by inhibition of the S-A node; establishment of independent ventricular or A-V rhythm due to inhibition of the S-A rhythm; and the occurrence of bundle branch beats, all from inhibition.

If the vagi are cut then the normal high rhythm persists with sequential beats that result in sustained blood pressure for a minute or so after respirations cease. The fast rate continues straight through the early period during which anoxemial inhibition occurs when the vagi are intact.

After a more or less prolonged period, 3 to 5 minutes following the respiratory pause, and when the blood pressure approaches zero and the heart beats can no longer be readily distinguished by the mercury manometer, then a second and direct disturbance of the heart rhythm occurs. There is great slowing of the rate, heart block and independent rhythms. There is loss of auricular rhythm due to reversed block or of ventricular rhythm from direct block. Finally complete cardiac pause ensues. This seems to be the stage observed by Mathison and the onset by his methods was more abrupt than we observed.

A difficulty in correlating these facts with those related in the literature depends upon the fact that Mathison and the others used rapid methods to induce asphyxiation. The method of occlusion of the trachea suddenly withholds oxygen and fails to remove carbon dioxide, as does also the rebreathing of pure nitrogen from a bag. Our method of rebreathing purified air progressively withdraws oxygen. The rate of withdrawal used by us allows the body tissues and organs to progressively adapt to the condition of oxygen lack. There is less danger from misleading secondary reactions. On the whole a truer picture of uncomplicated anoxemia seems to result.

Mathison and others do not record normal respirations when they do occur and it is difficult to determine from blood pressures alone the corresponding times in the asphyxial cycle. Mathison's experiment 5 shows a long period of large variations in the blood pressure and a slower heart rate induced at about 60 seconds after beginning nitrogen respirations. The high blood pressure and large pulse amplitudes suggest that this phenomenon can not be the late and final direct anoxemia described by us. We are at a loss to explain the difference unless the cat and dog show a fundamental variation in this regard. We refer in comparison to our figures 1, 18, 22 and 34.

Haggard (11) has recently studied carbon monoxide asphyxiation in which the blood changes and the electrocardiographic responses were observed in animals poisoned by carbon monoxide gas. He carried experiments to fatal terminations, also recovered animals after gassing. He atropinized animals but did not operate as a method of removing vagus influence.

Haggard did not take continuous electrocardiograms but his intermittent tracings show cardiac phenomena which at one time or another we have obtained, with the exception of ventricular fibrillation. His figures 3 to 9 and 11 to 13 contain complexes that are common enough pictures in progressive anoxemia as obtained by our methods. One could interpret his results as due to true anoxemia rather than due to carbon monoxide an interpretation on which Gasser and Loevenhart based their method for inducing anoxemia. In our experiments also "the cardio-inhibitory center maintains its activity longer than does the respiratory center." Haggard (p. 398) describes a phenomenon which he attributes to "fatigued cardio-inhibitory center." We obtained some not very conclusive evidence on this point. In the preceding pages we have given the facts and explanations which will clarify Haggard's observation that after atropine and carbon monoxide "the heart maintains a rapid rate until the time of respiratory failure. Following this, the rate slowed, the P-R time increased and A-V block developed, but without the stage of auricular cessation noted in the unatropinized animals." The statement could be made of dogs under anoxemia provided we considered only the very early and the final effects of anoxemia, pictures due to two very different causes. It is apparent that Haggard missed the beautiful sequence, probably true for monoxide asphyxia as well as for simple anoxemia, by not taking continuous electrocardiograms.

We deem it more than probable that the cyle of circulatory events is fundamentally similar by the various methods of producing anoxemia. The sequence and intensity of the reactions, however, must vary with the rapidity of the onset, and with the rate and thoroughness with which oxygen is removed from the tissues. In the last and final extreme reduction of cellular oxygen suppresses the fires of physiological processes. However resistant the tissue or organ may be its activity is smothered by oxygen want.

Protocol. Exper. 26, Dog 12, Wt. 10 K. Chloretone 0.3 gram per K., air allowance 3 liters, oxygen at the crisis 4.5 per cent. Electrocardiograms.

This experiment ran through a very even and uniform pre-crisis period showing a gradual use of oxygen and little or no variations of blood pressure until the 11th minute. Blood pressure then increased until the end of the 12th minute, which marks the first maximal pressure. There was great slowing and irregularity of the heart rate but normal sequence through the maximal. At 13 minutes, 30 seconds, respirations stopped. The heart rate was progressively slowed and the pulse amplitude greatly increased. At 13 minutes, 58 seconds, the rate became suddenly very slow and continued slow through about 80 seconds. Insufflation was then begun and after 10 very slow beats recovery occurred rapidly, at 15 minutes, 45 seconds. This point is marked in figure 1 by the letter *S* over the first normal or sequential contraction in the recovery.

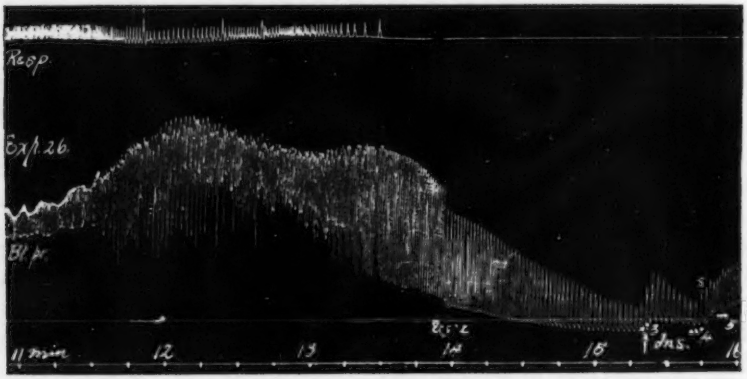


Fig. 1. Experiment 26. The blood pressure and respiratory records show a somewhat unusual form of response to anoxemia. The blood pressure crisis exhibits two periods of maximal pressure with profound slowing of respiration at the onset of the first and stopping at the second. At 13 minutes, 58 seconds, the heart beat suddenly drops to a slower rate. Plate I, figure 2, shows that this change is due to a shift from an S–A to an A–V rhythm. At 15 minutes, 20 seconds, insufflation was started. It had no influence on the electrocardiograms for 25 seconds when at 15 minutes, 45 seconds, regular sequential beats were reëstablished on the particular beat marked *S* (see also fig. 4). The 10 beats following insufflation arise from a rhythmic point in the left bundle branch. Electrocardiograms are presented of the individual beats marked by dots. Magnification × 0.56.

Continuous electrocardiograms beginning at 12 minutes were obtained (see plate I, figs. 2 to 5). The electrocardiograms showed slow and irregular rhythm but no abnormal sequences until 13 minutes, 58 seconds, when the rhythm shifted from an S–A to an A–V origin, figure 2. With the shift the Q wave appeared and was followed by an exceptionally tall R wave, 16 mm., in comparison with the normal sequential complex which in this animal showed an R of only 2 to 3 mm. At 15 minutes, 22 seconds, the origin of the rhythm shifted to a still lower point in

the A-V bundle system, figure 3. The complex from the new focus has an S wave of 10 mm. amplitude and a tall positive T wave. It is typical of left ventricular dominance but its type shows bundle origin. The focal center is apparently in the left bundle branch and remains there for the next 10 beats. After 10 beats insufflation introduced enough oxygen to bring about a normal sequential heart beat of increasing rate and final recovery.

This whole phenomenon is interpreted as vagal stimulation by anoxemia at the center. The most striking new observation is the fact that anoxemia affects the vagal center profoundly enough to drive the rhythm to a point so low as the left bundle branch.

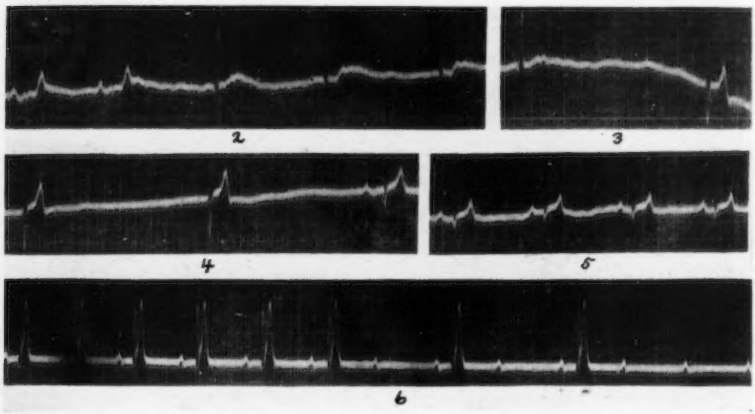


Plate I, Experiment 26. The positions of the electrocardiograms shown in figures 2 to 5 of this plate are marked in the blood pressure curve by dots under the corresponding beats. Figure 6 is the terminal record of experiment 36.

Fig. 2. Five complexes recorded at 13 minutes, 58 seconds, showing the shift in the rhythm from S–A to A–V origin. Extrinsic currents interfere but there is no very clear evidence of a P wave. Possibly the slight negative depressions in the T-waves of the last two complexes can be attributed to the auricle. There are 49 complexes in this group. Definite and clear reversed conduction characterizes the last 29.

Fig. 3. At this point A–V rhythm with reversed conduction suddenly ceased and a ventricular complex characteristic of left ventricular dominance and bundle branch origin began. This type runs for 10 successive contractions. These contractions are explained on the assumption of origin of the beat in the base of the left bundle branch.

Fig. 4. The two complexes of left bundle branch origin are followed by one sequential beat. Sequential contractions continued until complete recovery of normal rate and conduction. The sequential beats have at first a relatively long P–R interval but conduction slowly improved under insufflation.

Fig. 5. Fifth to eighth sequential beats during the recovery under insufflation.

Fig. 6. This excerpt from a continuous electrocardiographic record of experiment 36 through 7 minutes after respirations stopped shows the direct asphyxial effect on the heart when the vagi are cut. The rate progressively slowed to the auricular rate shown in this figure, 14 minutes. Then there occurred 2–1 block for two periods followed by complete block. During the last minute and a half of the entire record six independent ventricular complexes occurred. When the electrocardiographic record ceased the auricular rate was still 25 per minute and regular.

Protocol. Exper. 29, Dog 13, Wt. 9 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen at end 2.2 per cent. Vagi intact. Dog not revived. Electrocardiograms through the early and the beginning of the late anoxemial state, plate II, figures 8 to 16.

Anesthesia relatively light, occasional skeletal muscle contractions during the early stages of the experiment. Respirations rapid at the beginning but very slow and irregular from the 4th to the 8th minute and regular and typical during the last portion of the test.

Blood pressure was more sensitive to external or reflex stimulation than usual. There were two maximal pressure waves separated by 1.5 minutes, figure 7. From 13 minutes, the heart progressively slowed until at the last respiration the pulse amplitude was 70 mm. From the moment of the last respiration blood pressure fell uniformly through 2.5 minutes, when the heart beats were no longer strong enough to record. The heart rate remained uniformly slow through 70 odd seconds, then gradually increased in rate but still decreased in amplitude. At 16 minutes, the pulse cannot be counted on the blood pressure tracing, though it is clear and sequential in the electrocardiograms.

There were four respiratory gasps after regular respirations stopped. The second and third are followed by a slight increase in heart rate.

The electrocardiograms showed the usual normal—P 2 mm., Q slight, R 21 mm., S none, T negative 4.5 mm., P–R 0.098 seconds, R–T 0.200 seconds, rate 140 per minute.

At 12 minutes, 7 seconds, the T wave became positive. At 12 minutes, 23 seconds, figure 2, the T wave had increased to 6.5 mm. At 12 minutes, 53 seconds, the T had reached an amplitude of 12 to 14 mm. At about 13 minutes, 30 seconds, the slowing is more pronounced, and at 13 minutes, 42 seconds, S–A rhythm was inhibited and A–V rhythm established, figure 11, plate II. The type of reversed conduction shown in the third complex of figure 11 continues through 9 beats after which for 23 consecutive beats there was no evidence of auricular action. The 24th and 25th beats, the 3rd and 4th of figure 13, are sequential. At this point the first respiratory gasp shown in figure 7 occurred. These are followed by 10 beats with no P wave in evidence.

On the last complex of figure 13, the character of the complex changes. There is now a very short R wave, deep and profound S, and a continued tall T. This is a typical left ventricular dominance. This we also explain by assuming that at this point the origin of the rhythm shifted down the A–V node to a still lower point in the conducting system, to the left bundle branch. This type of beat gradually shifted back to the normal sequential beat as shown in figure 14. The first complex in figure 14 introduced reversed conduction. In the third complex

the P wave occurred before the ventricular complex, and in the fourth and fifth the regular sequential beats appeared and continued for some 12 beats before a second respiratory disturbance occurred. The third complex in figure 14 shows left ventricular dominance but an auricular beat occurs higher up either in the tail of the S–A node or in the atrial groove. Possibly the left ventricular type in this case can be attributed to relative right bundle block rather than left bundle rhythm. If so, it would indicate an influence of the vagus nerve on conduction extending well down into the ventricular portion of the bundle system and greater on the right bundle.

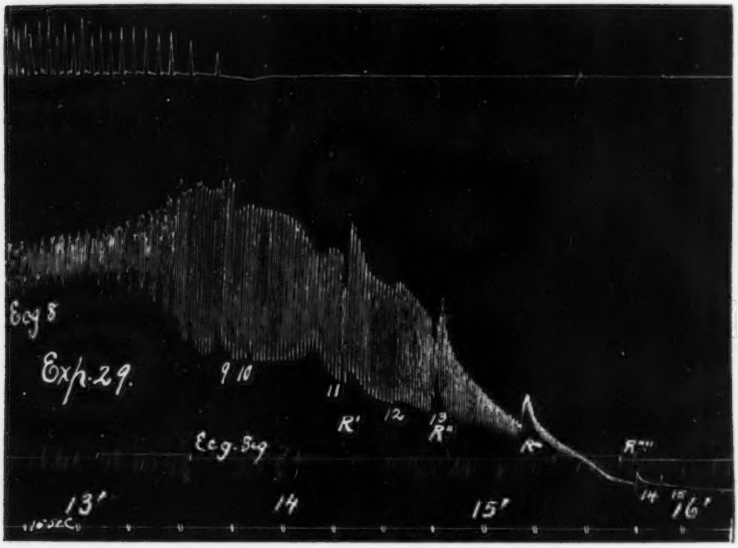


Fig. 7. Experiment 29. The respiratory and blood pressure tracings at the terminus of the test. Top line respiratory movements which ceased at 13 minutes, 44 seconds. Middle line blood pressure. The maximum or crisis occurred at 13 minutes, 30 seconds. The numbers below the blood pressure tracing indicate points figured in the electrocardiograms, plate II, figures 10 to 17 inclusive. R', R'', R''', R'''' respiratory gasps occurred after rhythmic respirations had ceased. No attempt at recovery. Magnification × 0.68.

Sequential beats continued from 14 minutes, 30 seconds, through to 15 minutes, 50 seconds, when partial 2–1 block occurred as shown in figure 16. In the mean time the duration of the P wave and the length of the P–R interval very progressively increased to the extreme degree shown in figure 16 when 2–1 block was established. The electrocardiographic record ceased at 16 minutes with the type of record shown in figure 17, 2 minutes, 20 seconds, after respirations ceased.

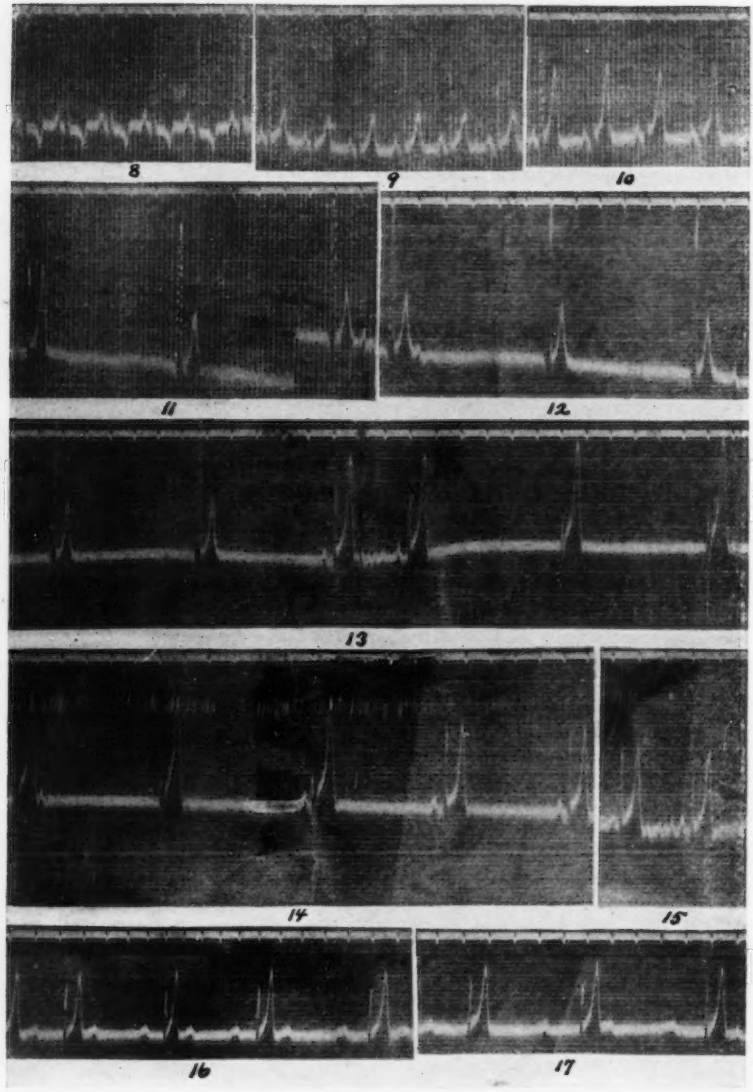


PLATE II

Plate II, Experiment 29.

Fig. 8. Normal electrocardiograms. Note the characteristic tall R and the negative T waves.

Fig. 9. Time, 12 minutes, 23 seconds. The T–wave had changed from a negative to a positive 16 seconds earlier. R now decreasing.

Fig. 10. Time, 12 minutes, 53 seconds. Both the R and the T waves progressively increased between figures 9 and 10.

Fig. 11. Time, 13 minutes, 42 seconds. Rapid inhibitory slowing of the rate (vagal) during the preceding 10 beats. S–A rhythm inhibited and A–V established at this point. Reversed conduction continued through 9 beats with block on the second, third and ninth between this and figure 12.

Fig. 12. Time, 13 minutes, 56 seconds. Reversed conduction blocked in the second complex and permanently blocked in a series of complexes following this point. Note the delay of reversed conduction in the third complex compared with the first in this figure.

Fig. 13. Time, 14 minutes, 15 seconds. Transitions occur in the pacemaker along the A–V node ending in a ventricular beat with left dominance in the last complex. The third and fourth complexes occur at the first anoxemial respiratory gasp, shown in the blood pressure curve, figure 7, and are due to momentary but partial suppression of the vagal inhibition.

Fig. 14. Time, 14 minutes, 30 seconds. Sudden transition from deep A–V rhythm to a normal sequential but slow rhythm. From this point no further irregularities in sequence occur until permanent block appeared 80 seconds later.

Fig. 15. Time, 14 minutes, 45 seconds. Momentary auricular flutter at the second respiratory gasp shown in figure 13. Sequence normal when it occurs.

Fig. 16. Time, 15 minutes, 50 seconds. Appearance of direct anoxemial block to 2–1 rhythm. For the preceding 30 seconds the P–R became progressively longer, from 0.12 second, to 0.28 second. The duration of the auricular contraction also progressively increased as shown in this figure.

Fig. 17. Time, 16 minutes. Establishment of permanent block but with both S–A and A–V rhythms still occurring. The record not taken beyond this point.

The reëstablishment of sequential beats after the extreme inhibition shown in the first anoxemial slowing indicates a partial escape from the vagal inhibition of conduction. Henderson and Haggard have given evidence indicating a similar phenomenon of escape after carbon monoxide asphyxiation.

Protocol. Exper. 33, Dog 16, Wt. 7.5 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen reached 4.37 per cent. Vagi intact. Electrocardiograms throughout the critical asphyxial stage, plate III, figures 19 to 21.

An excellent record of respirations and blood pressure was obtained with unusual features in the terminal phase. Electrocardiograms continue through the entire critical post-crisis period, figure 18, and plate III, figures 19, 20 and 21. The respiratory record shows a very uniform consumption of oxygen to 10 minutes, a progressive falling off of oxygen used until respirations ceased at 12 minutes, 20 seconds.

The rise of blood pressure was sharp during the crisis, notwithstanding the fact that the heart rate slowly decreased from 160 at 10 minutes, 40 seconds, to

128 at the time of maximum pressure, 12 minutes. There are four periods of pronounced cardiac slowing during the 13th minute, the first occurring between the last two respirations, figure 18.

At the beginning of the 4th pronounced period of slowing, marked *A–V rhythm* on the figure, auricular contractions disappeared leaving a pure ventricular complex. Occasionally there was reversed conduction with a rather long R–P interval, figures 20 and 21. Beginning with the last complex in figure 21, sequential rhythm was established, and the rate increased as shown in the blood pressure tracing, figure 18. This was possibly a release from the vagus anoxemial inhibition on account of the entrance of air obtained through insufflation begun at 13 minutes, 55 seconds. However, no recovery of the animal occurred.

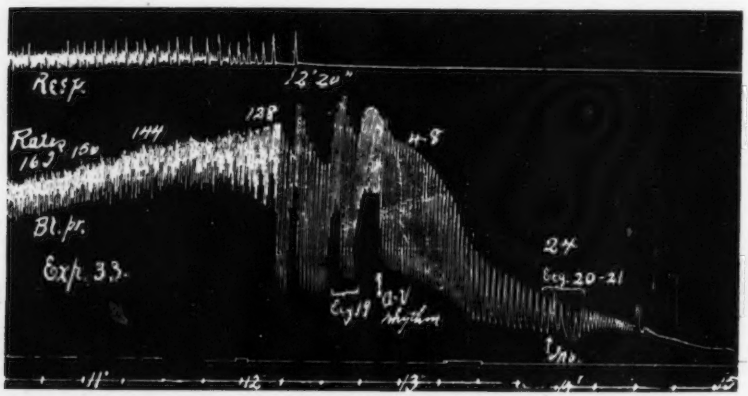


Fig. 18. Experiment 33. The terminal stage of experiment 33, vagi intact. The numbers above the blood pressure curve are heart rates per minute. At the blood pressure crisis three great irregularities in the heart rate appear, i.e., three groups of slow rates each followed by a momentary recovery. These periods are in reality 2–1 blocks as shown by the electrocardiograms, plate III, figure 19. *Ecg. 1* and *ecg. 2–3* are figured in plate III. *A–V rhythm* marks the point where the S–A rhythm was completely inhibited. *Ins.*, insufflation began. On the fifth beat, the last complex of figure 21 of the electrocardiographic series, normal S–A rhythm was reëstablished. Magnification × 0.59.

Protocol. Exper. 36, Dog 18, Wt. 10 K. Chloretone and ether, air allowance 4.5 liters, oxygen at the end 3.46 per cent. Vagi cut at the beginning. Dog not revived. Continuous electrocardiograms for 6 minutes, beginning 10 seconds before respirations ceased, plate I, figure 6.

Respirations very irregular, rather rapid until the last minute when they slowed down at the anoxemial crisis.

The blood pressure increased at the moment both vagi were cut at the beginning of the experiment and remained high until anoxemia appeared. The pressure then very gradually decreased with failing respiration. No slow beats at

the crisis, very regular heart rate with gradual decrease in pulse amplitude until the variations were no longer recorded by the manometer, figure 22. Sequential heart beats to 14 minutes, 30 seconds, plate I, figure 6. At 4 minutes, 5 seconds, after respirations stopped, complete block occurred. The auricle continued to beat with regular but decreasing rhythm for 7 minutes, 35 seconds, after respirations ceased. The auricular rate was 25 per minute at this time when the electrocardiographic record was stopped. The development of heart block in this experiment was like that in experiment 40, plate IV, figure 32.

In this experiment conduction was first eliminated in the late anoxemial asphyxiation of the tissue as in experiment 40. The electrocardiograms show that the inception of block was preceded by a group of rapidly lengthening P–R intervals. The P–R intervals in the complexes figured are 0.200 second, 0.200, 0.200, 0.208, 0.212, 0.220, 0.232, block, 0.228, block now complete. Six irregular and independent ventricular complexes occurred late after the development of block.

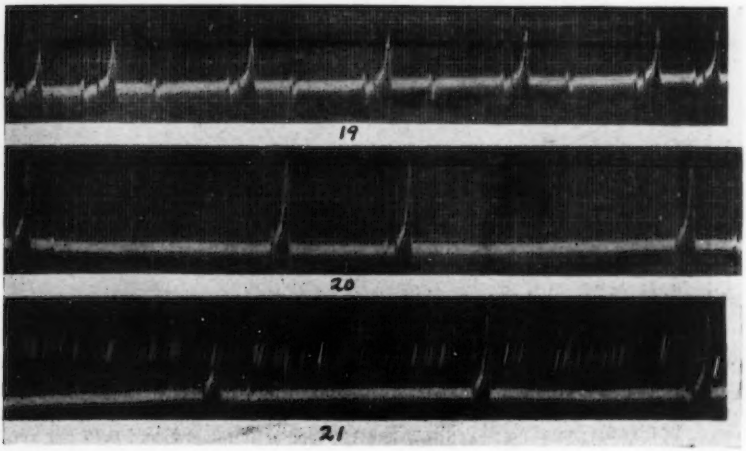


Plate III, Experiment 33.

Fig. 19. This figures the third group of four beats at a slow rate as shown in the blood pressure curve of experiment 33. Four auricular contractions are shown to be blocked and a 2–1 rhythm occurs. Recovery of conduction extended through the succeeding rapid period shown in the blood pressure curve.

Fig. 20. An A–V rhythm has persisted through the preceding 70 seconds. Reversed conduction occurs occasionally only. It is shown in the first and fourth complexes of this figure. Reversed block occurs in the second complex. The third complex is produced by an escape to S–A rhythm at the moment when insufflation began, see the blood pressure curve, figure 18.

Fig. 21. Continuation of figure 20, showing reversed conduction, block, and the permanent return of S–A control beginning with the last complex. From this point on sequence is normal and the rate progressively increases.

Protocol. Exper. 38, Dog 19, Wt. 9 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen at crisis 2.38 per cent. No electrocardiograms. Respiratory and blood pressure curves.

Respirations rapid, use of oxygen uniform, but decreasing at the very last before respirations ceased at 14 minutes, 16 seconds.

The rise of blood pressure was moderate at the crisis. Heart rate at its maximum at 13 minutes, 30 seconds, near the crest of maximal blood pressure. The heart slowing began about 30 seconds before the respirations ceased, became very profound at 14 minutes, 35 seconds, with a rate of 44 per minute. The right

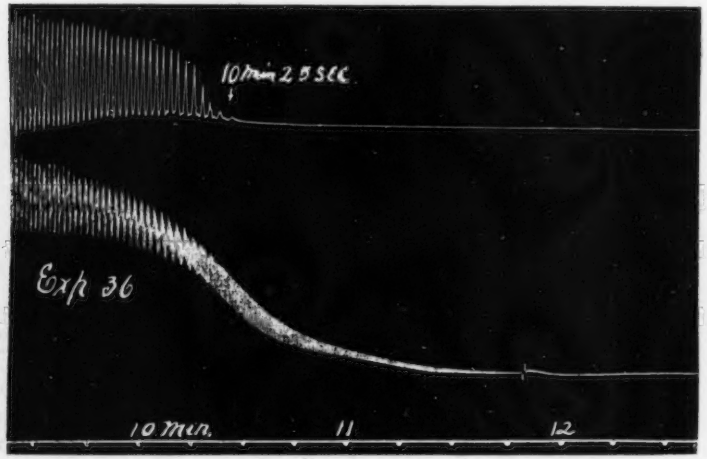


Fig. 22. Experiment 36. Showing the terminal respiratory and blood pressure records of a comparatively infrequent type of response to anoxemia when the vagi are cut at the beginning of the test. Respirations ceased at 10 minutes, 25 seconds, while the dog was inspiring 3.62 per cent oxygen and 0.45 per cent carbon dioxide. The blood pressure declined earlier than the rule but the heart rate was sustained in normal sequence for 3.5 minutes and the auricles still contracted at the end of 17.5 minutes when the record was discontinued. Figure 6, plate I, shows the beginning of direct asphyxial heart block at 14 minutes. Magnification × 0.76.

vagus was cut at 14 minutes, 58 seconds. The rate immediately doubled in partial release. The left vagus was cut at 15 minutes, 40 seconds. At this point the rate was released to 216 per minute, a greater rate than at the maximal blood pressure. During the interval between the cutting of the right and left vagus nerves the blood pressure was relatively high and the pulse amplitude great (see fig. 23).

Artificial respirations were established before anoxemia had advanced to the second asphyxial stage, natural respirations returned at 17 minutes, 15 seconds.

Protocol. Exper. 40, Dog 20, Wt. 16 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen at end 2.5 per cent. Electrocardiograms. Vagi cut during the cardiac slowing following the respiratory crisis. Insufflation but no recovery.

The blood pressure was very uniform and even until the 6th minute when the pressure began to rise and the heart rate to increase. The maximum pressure was reached at the moment when respirations stopped, although the average high pressure was maintained one minute and more longer.

Between the last two respirations 22 heart beats occurred. Following the last respiration there are 56 beats to the point marked *left vagus cut*, figure 24. These groups are each slower than the preceding. At the last group of 12 beats the blood pressure was 158 and the pulse amplitude 80. When the left vagus was cut slow swinging pulses occurred to the point marked *R. V. cut*. There are four slihgt

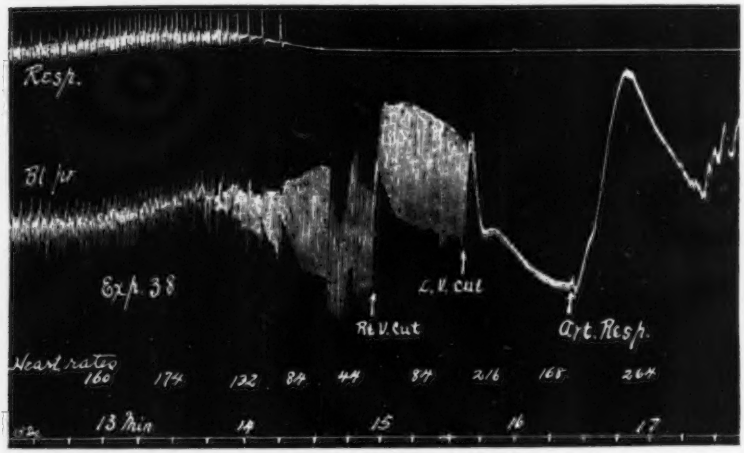


Fig. 23. Experiment 38. Slowing of the heart rate began at 13 minutes, 40 seconds, and at 14 minutes, 30 seconds, had dropped to 44. The right vagus was cut at the point marked. There was an immediate increase of the rate to 88 per minute. When the left vagus was cut the rate immediately escaped to the precrisis figure and rapidly ran up to 216 per minute. No electrocardiograms were obtained. The heart rates are given on the tracing. Magnification × 0.54.

irregularities in this series, otherwise they are remarkably even, though the pulse amplitudes progressively decreased. Counting the four irregularities there are 62 beats in the interval. When the right vagus was cut the pressure was 122 with pulse amplitudes of 68. Instantly the heart rate increased and the pressure struck a maximum of 130 rapidly falling to 108 in 8 or 10 seconds. After a small group of very irregular pressures a very regular series of heart beats and even pressure variations occurred through 25 seconds, the pressure at the beginning averaging 86. At the end of this regular group the pressure was 76. Insuf-

flation then produced irregularities in the blood pressure which however continued to fall. No recovery was obtained.

The normal electrocardiogram did not vary from the usual type. The R was tall, 23 mm., and T diphasic with the negative wave moderate. This type continued through the records of the 4th and 8th minutes. Continuous electrocardiograms began at 8 minutes, 55 seconds, and ran through the entire post crisis. At the beginning of the continuous record the T wave was positive, 9 mm. in amplitude. By counting the regular beats corresponding to the first, second and third blood pressure groups preceding the cutting of the left vagus, it was easy to identify the irregularities in the electrocardiograms. They are associated with a series of progressive shortenings of the P–R intervals. Measuring straight through the three irregular periods shown in the blood pressure record before the left vagus was cut we have the following P–R times in order: 1st beat,

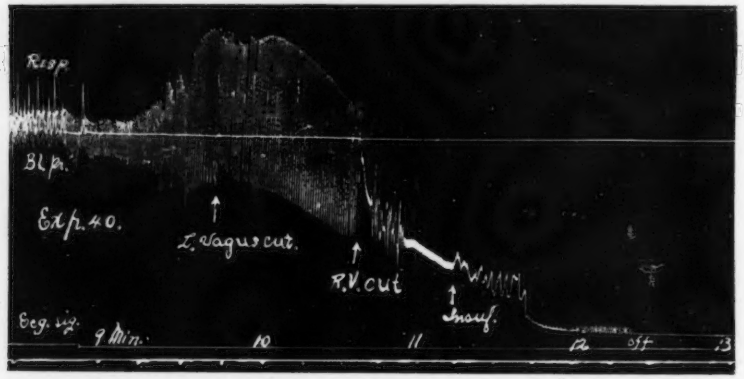


Fig. 24. Experiment 40. Respirations stop at 8 minutes, 56 seconds. Fifty seconds later the left vagus was cut. At 10 minutes, 35 seconds, the right vagus was cut. *Insuf.*, insufflation began but changed to bellows and stopped at *off*. No recovery. The electrocardiographic record was continuous from 8 minutes, 54 seconds, to 17 minutes, 40 seconds. At 12 minutes, 50 seconds, independent auricular and ventricular rhythms, i.e., complete block was established. At 14 minutes, 40 seconds, the auricular electrocardiograms were still regular but became too weak to photograph. At 17 minutes, 40 seconds, the ventricle was contracting irregularly at about 6 per minute. The record was then stopped at 7 minutes, 46 seconds, after natural respirations ceased. The time line is raised to 30 mm. pressure. Magnification × 0.59.

0.112 second; 2nd, 0.100; 3rd, 0.092; 4th, 0.080; 5th, 0.084; 6th, 0.064; 7th, 0.072; 8th, 0.052; 9th, 0.012; 10th, 000; 11th, 0.128; 12th, 0.100; 13th, 0.112; 14th, 0.108; 15th, 0.092; 16th, 0.052; 17th, 000; 18th, 0.136, 19th, 0.116; 20th, 0.100; 21st, 0.088; 22nd, 0.056; 23rd, 0.020; 24th, −0.040 (reversed conduction); 25th, 0.112; 26th, 0.092; 27th, 0.088; 28th, 0.084; 29th, 0.120. These conduction times identify the irregularities as due to a progressive displacement of the rhythmic center in the

descending direction the most striking in our series. The 10th, 17th and 24th are the critical complexes, plate IV, figure 28.

At the point marked, plate IV, figure 29, the first or left vagus was cut. The two beats preceding the section of the nerve have buried P waves, so also the first beat following the cut. The third complex shows a well-marked reversed conduction, the P wave occurring late in the T. This auricular complex began a series of negative P waves running through the entire group of electrocardiograms until the second or right vagus was cut. The 5th beat showed a reversed conduction time of 0.216 second, in the sixth beat the R–P interval is 0.328 second, if indeed this P should not be considered as belonging to the following complex. The next 5 or 6 contractions introduce variations of similar type, and this phenomenon recurred at intervals until the second vagus was cut. Certain ventricular complexes show no associated auricular contractions.

At the intervals between the 58th and 63rd beats after cutting the left vagus, there are variations in the iso-electric period which mark the lifting and cutting of the right vagus nerve. Although the nerve was cut promptly the exact point of cutting is in doubt. The 64th beat is partially recorded only. The 65th beat and the series that follow are normal sequential complexes increasing very rapidly in rate and decreasing in the amplitude of the T, through 18 or 20 beats. The 18th recovery beat is at a rate of 242, P–R 0.104 second, R–T 0.104 second, P 1.6 with rather broad base, Q 1, R 17, S none, and T 7. After the 22nd beat there was some irregularity in the sequence.

For about 40 seconds following the section of the second vagus the record was regular and uniform with slight broad wavelike variations (suggestive of some extrinsic influence). At the stage of anoxemia when these end the T waves greatly augment, changing from 6 to 10 mm. in about 10 beats. The P–R interval lengthens to 0.14 second. At the end of the tracing the T wave had increasd to 16 mm. and the P–R to 0.16 second and the rate had slowed to 106 per minute. These complexes are regular and slow sequential beats with tall T waves and increasingly long P–R intervals. The 10 complexes preceding complete and final block have P–R intervals that measure 0.136 second, 0.152, 0.152, 0.160, 0.160, 0.168, 0.220, 0.232, block, and 0.248. All succeeding contractions are blocked, figure 31. A regular auricular rhythm continued through about 2 minutes but the P waves became increasingly faint until they could not be distinguished at 14 minutes, 50 seconds.

Occasional ventricular complexes occur during this time. The first one is fused with an auricular complex, the second, third and fourth are obviously independent, and fifth appears so but follows a P wave by 0.092 second, the 6th and 7th follow P waves by 0.180 second, the 8th by 0.008 second, and in the 9th P is buried in the ventricular complex. The ventricular rhythm is obviously wholly independent.

The auricular rate dropped from 100 to about 26 per minute during the time of block. Ventricular complexes only are visible throughout the electrocardiographic records of the 5th and 6th minutes after respirations ceased. These are at a low but fairly regular rate, about 12 per minute at first but 6 per minute in the tracing which closes our record, figure 33.

The disturbances following the stopping of respiration can in this case all be attributed to vagospasm. They disappear on cutting both vagus nerves. The

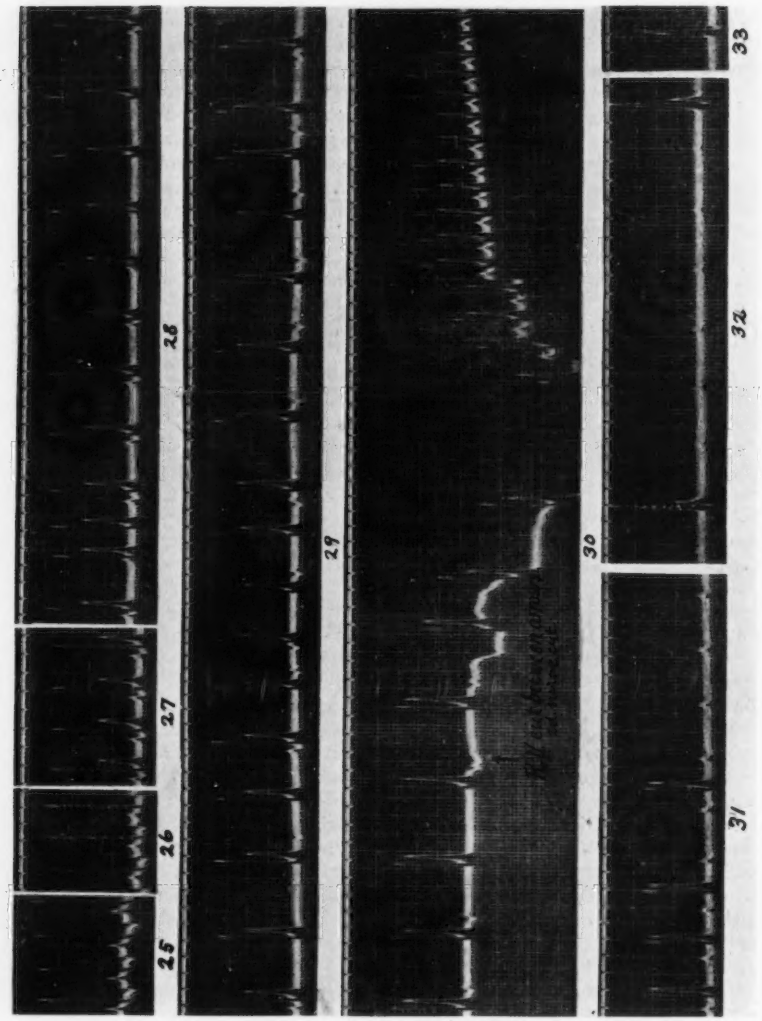


PLATE IV

sequential rhythm that returns is perfectly comparable to that of experiments in which the vagus nerves were cut before anoxemial asphyxiation began. In this experiment when the heart itself became asphyxiated sequential beats were suddenly stopped by block. The auricle continued in regular rhythm through many seconds until finally the auricular complex became too faint to be distinguished. In the mean time independent ventricular complexes at long but comparatively regular intervals appeared and persisted to the end of our record. The appearance of augmented T waves with the onset of the period of slowing of the heart from cardiac tissue anoxemia was typical of the course of other experiments.

Insufflation used late in the test when blood pressure was low but while normal electrocardiograms were running was unsuccessful toward reviving the animal, in fact had no observable effects.

Protocol. Exper. 41, Dog 19, Wt. 19 K., Chloretone 0.3 gram per K., air allowance 4 liters, oxygen reduced to 1.8 per cent, electrocardiograms, blood pressure, vagi cut at the crisis.

Respirations comparatively regular for 6 minutes then rapid and irregular to 7 minutes, increasing rate and amplitude to 9 minutes, decreasing amplitude 9 to 11 minutes, decreasing rate 10 to 11 minutes. Respirations cease at 11 minutes. For the first 6 minutes deeper individual inspirations occur about every 6th respi-

Plate IV, Experiment 40.

Fig. 25. Normal electrocardiograms, showing negative T waves.

Fig. 26. Seven minutes, 30 seconds, from the beginning of the anoxemial test.

Fig. 27. Eight minutes, 55 seconds, from the beginning of anoxemia. End of respirations. T wave became positive at 8 minutes.

Fig. 28. Nine minutes, 30 seconds. Periodic inhibitory displacement of the rhythmic center in the descending direction, each period ending in apparent block but probably buried P waves.

Fig. 29. The left vagus was cut at the mark *L. V. cut*, 9 minutes, 50 seconds. In the third complex the P is inverted and conduction is retrogressive. From this point through the interval before the cutting of the second vagus the P wave was always inverted, conduction was reversed and occasionally there was reversed block.

Fig. 30. At 10 minutes, 35 seconds, the second or right vagus was cut somewhere between the points marked, probably at the second arrow. After a few beats normal sequential rhythm was rapidly reëstablished, the rate increasing through the first 10 or 15 contractions following the second arrow.

Fig. 31. At 12 minutes, 50 seconds, or 2 minutes, 15 seconds after both vagi were sectioned, complete anoxemial block appeared. The auricle continued to beat in regular rhythm from the S–A center but the ventricle ceased beating. After a long interval occasional independent ventricular complexes appeared with increasing frequency.

Fig. 32. Thirteen minutes, 50 seconds. The ventricle now contracted at the rate of 14 to 15 per minute. The auricular rate was about 90. One minute later the auricular complexes were too weak to record.

Fig. 33. The last recorded ventricular complex, 17 minutes, 40 seconds, after respirations ceased.

ration, from 6 to 8 minutes fewer deep inspirations, from 8 to 10 minutes more frequent deep gasps that become very marked near the end at 11 minutes.

The blood pressure was very uniform and even, one of the most regular records of the series. After 5 minutes it slowly and progressively increased to a maximum at 10 minutes, 25 seconds. The maximum pressure came about 40 seconds before respirations stopped but after a decrease in the use of oxygen was apparent. The blood pressure fell very slightly through 40 seconds, then somewhat more rapidly until the vagus nerves were cut (see fig. 24). Later the pressure fell promptly to the level shown in the figure. The events are figured through only 4 minutes after respirations stopped.

The heart rate began at 109 per minute. In the 5th minute it had increased to 118, 121 in the 8th, 156 in the 10th, and 161 at 10 minutes, 15 seconds. The rate rapidly fell then to 145 and finally 44 when the right vagus was cut. There were no changes in heart rate between the cutting of the right and left vagi. When the second or left vagus was cut the rate immediately increased to a maximum of 185, then decreased through the rates shown in the figure to 64 at 14 minutes, 40 seconds, after which the manometer no longer recorded, though the electrocardiograph recorded complexes for 30 seconds more, when the heart stopped completely as shown in plate V, figure 42.

When the second vagus was cut the blood pressure immediately increased, then dropped again in 5 seconds, figure 34. This was followed by a slight second rise in pressure, then a progressive decline through 2 minutes, 25 seconds, when the heart beats were no longer visible on the manometric record. The heart rates through this period were as follows: 15 seconds before the vagus was cut 11 beats, and by 15-second periods after cutting, 44, 40, 40, 40, 36, 30, 24 and 20 on the 9th period but for the 10th not visible.

The continuous electrocardiographic tracing, beginning at 8 minutes, 45 seconds, shows beside the cardiac complexes certain gross waves corresponding to the respiratory rhythm. Periodically these waves are larger and check with the recorded deep sighing inspirations shown in the respiratory record. They aid in marking the end of active respirations in the rapidly moving electrocardiographic film.

The normal electrocardiograms show the following type: the rate is relatively high, 109 per minute. The P wave is positive and sharply defined, the P–R intervals average 0.14 second. The ventricular complex begins with a sharp abrupt R wave of short duration and large amplitude. There is only a slight S wave. The T is diphasic, with a sharp negative deflection which ends in an abrupt positive, the two of about equal amplitude. The duration of the R–T interval is 0.24 second.

At 8 minutes, 45 seconds, the rate had increased to 150 with a shortening of the P–R interval to 0.112 second. At 9 minutes, 30 seconds, the rate was 156, P–R time 0.110 second. The R had increased in amplitude to 20 mm. against the normal of 17. The heart rate reached its maximum of 161 at 10 minutes, P–R 0.096 second, R–T 0.21 second.

At 10 minutes, 30 seconds, the heart rate was 159. P–R now 0.088 second, the shortest conduction interval shown in the tracing. The T wave was no longer diphasic but terminated in a sharp positive of 5 mm. From this point on until respirations stopped the T wave gradually and progressively increased in ampli-

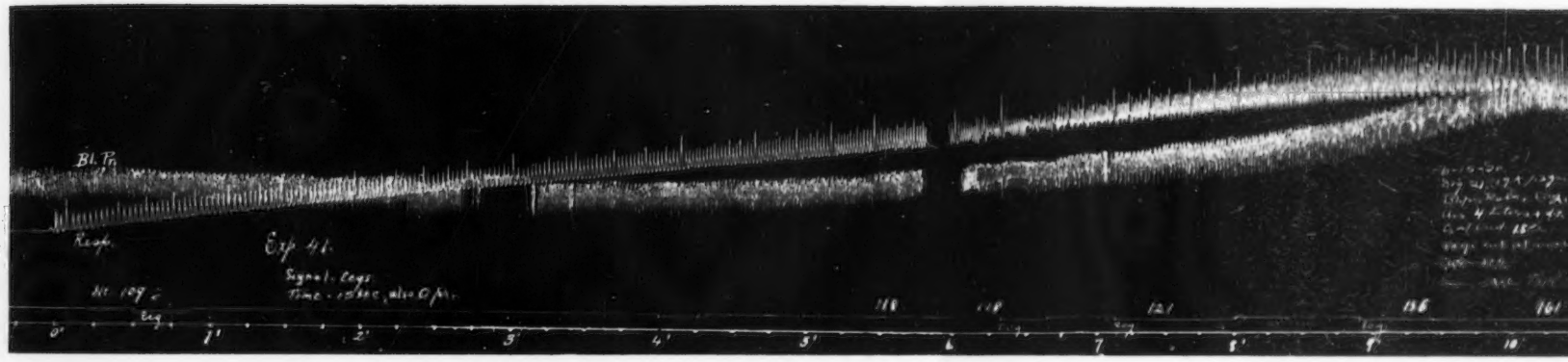
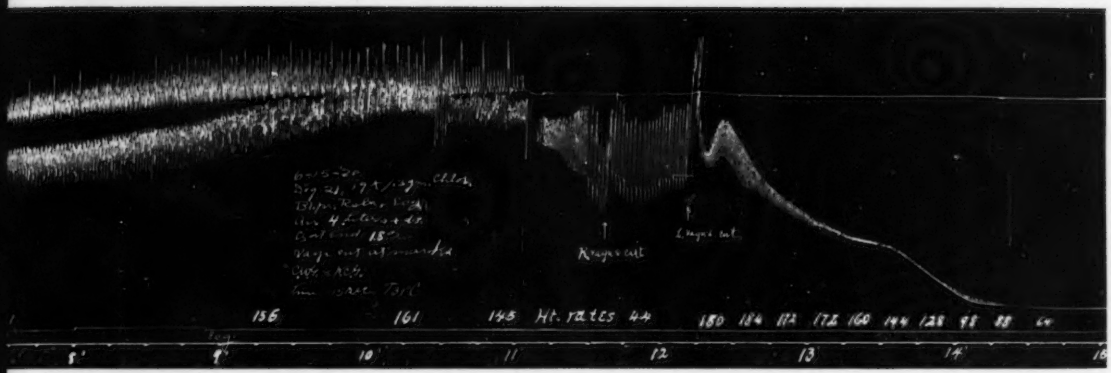


Fig. 34. Experiment 41. Blood pressure and respirations through the entire experiment. Time in 15 second intervals. Heart rates recorded just above the electrocardiogr in succession. No artificial respirations of any kind. Note the uniform rate of oxygen consumption until the anoxemial crisis approached. The original reduced one-half.



rates recorded just above the electrocardiographic signal line. At the points marked, the right and left vagi were cut approached. The original reduced one-half.

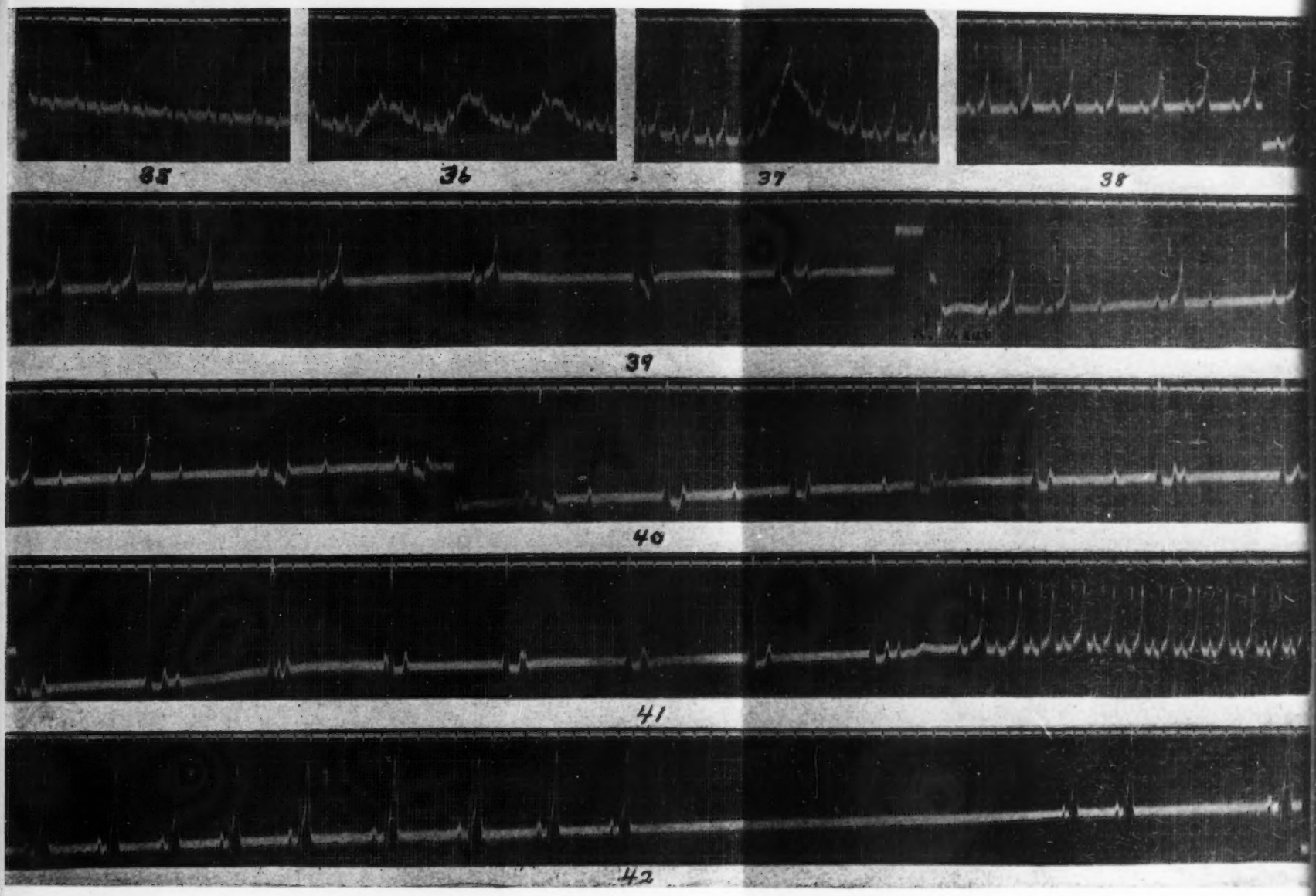


Plate V, Experiment 41.

Fig. 35. Normal electrocardiograms. The usual diphasic T wave.

Fig. 36. Time, 10 minutes, 5 seconds, from the beginning. The large waves are due to the influence of respiratory movements. The T wave now positive.

Fig. 37. Time, 10 minutes, 50 seconds. The T wave still further increased. The extrinsic currents are associated with the last respiratory gasp.

Fig. 38. Time, 11 minutes, 10 seconds. Already the inhibitory slowing of the heart is rapidly approaching. The electrocardiograms are remarkably uniform in type.

Fig. 39. Time, 11 minutes, 30 seconds. The right vagus cut at the point marked. The first five complexes are sequential. In the sixth and seventh the rhythm arises in the A–V node. In the sixth the P coincides with the T but falls later in the seventh, and conduction is reversed in both complexes. When the right vagus was cut at the point marked *R–V cut*, 5 sequential beats immediately recur, followed by 5 groups of 2–1 block, the last showing at the beginning of figure 40.

Fig. 40. Time, 11 minutes, 50 seconds, at the point marked. The 2–1 rhythm at the beginning of this figure quickly passes to a complete block. The third ventricular complex we interpret as arising in the A–V node as do the successive complexes in the remainder of this figure and in figure 41.

Fig. 41. Time, 12 minutes. The left vagus was cut at the point marked. Before the vagus was cut both auricular and ventricular complexes were present but independent. Immediately on cutting the left vagus, the second, the heart began sequential beats at a rapid rate and with P–R intervals shortening down for the first few complexes.

Fig. 42. Fifteen minutes, 10 seconds. This figure illustrates the last 12 complexes in the continuous series of 400 following cutting of the left vagus. There is no appreciable change in the P–R and R–T intervals at the last, the R waves progressively decrease and the S increases from the types in figure 41 to these shown here, and the T waves augment. Compare figures 6 and 33 in which auricular complexes persist after conduction is blocked by direct asphyxiation.

tude to a maximum of 11 mm. at 11 minutes, 20 seconds, 4 or 5 seconds after the stopping of respiration. Beginning at 11 minutes, the rates computed from 10-second intervals are 145, 109 and 44. These changes in rate are accompanied by an increase in the conduction time as shown by the longer P–R intervals. The R wave decreased in amplitude through 19, 18 and 15 mm., respectively. During the 30 seconds of progressive slowing of rate the blood pressure fell. There was a corresponding increase in the pulse amplitude.

The first or right vagus nerve was cut at the point marked in figure 39, plate V, 11 minutes, 35 seconds. There were five slow swinging pulses just before the nerve was cut. Inhibition increased until the S–A rhythm gave place to an A–V rhythm, as shown in the last three complexes before the right vagus was cut. The last complex shows reversed sequence, the auricle contracting in response to A–V rhythm as in the human (2) (fig. 9, plate I). When the first or right vagus was

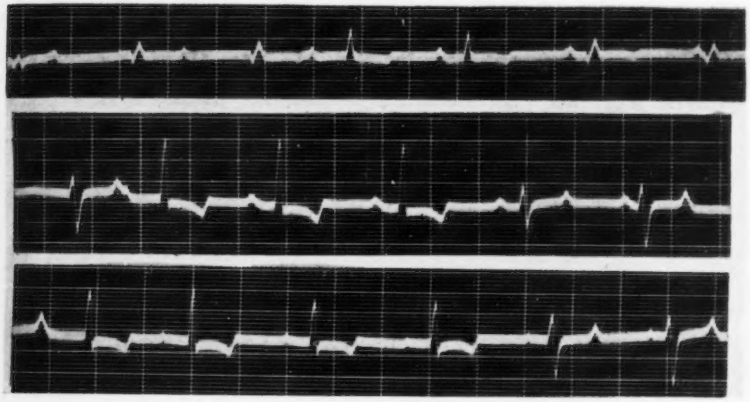


Plate VI, figure 43. Electrocardiogram showing the displacement of the pacemaker to the left bundle branch in a dog under the influence of morphine. This experiment was obtained by Dr. Frank N. Wilson and Dr. George R. Hermann, by whose kind permission it is here reproduced. The three conventional leads are shown.

cut there was temporary release from inhibition to a faster rate and normal sequential beat. After two beats a 2–1 rhythm returned for five or six groups before complete block occurred.

The ventricular rhythm during the vagospasm was very regular, rate 44. The auricular rate was absolutely irregular. The P wave was positive throughout but the P–P intervals have no regularity and cannot be lined with the ventricular complexes during this time. The intact left vagus does not inhibit the S–A nodal rhythm but it does block conduction.

On cutting the second or left vagus at 12 minutes, 10 seconds, the normal sequential type of electrocardiograms immediately returned, figure 41, plate V. The return rate was greatly augmented during the first few beats. This was

without change in the P–R and R–T intervals but with a tremendous increase in the amplitude of the T wave.

Sequential beats after sectioning the second vagus ran a continuous series for 400 consecutive beats before rhythm suddenly ceased as shown in plate V, figure 42. During this series the rate progressively decreased. The electrocardiograms were remarkably regular and uniform in character. However one striking phenomenon recurred here, namely, the augmentation of the T wave as direct cardiac anoxemia advanced. This phenomenon begins in this test early, by the reversal of the normal initial negative T. The amplitude rapidly increased at about the time respirations stopped. The T wave took on the tall type characteristic of A–V nodal rhythm during the vagospasm. When the second nerve was cut the T waves were at once almost as tall as the R waves and became taller to the end while the R waves progressively decreased.

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INFLUENCE OF BLOOD SERUM ON THE COAGULATIVE ACTIVITY OF TISSUE EXTRACTS

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While attempting to develop an anti-serum for tissue fibrinogen (thromboplastin) and for our tissue anticoagulant (protein fraction of tissue fibrinogen) we happened on the unexpected discovery that normal rabbit serum possesses in a very marked degree the property of rendering more intense the coagulative action of tissue extracts. This increase (being as high as about thirty-fold in one case) was only temporary, later giving way to a decrease in coagulative activity, but was so striking while it lasted that it led us to wonder whether it might not be of considerable interest in regard to the intravenous injections of supposedly non-toxic substances.

There has been considerable dispute in the past as to the action of serum on organ extracts, some claiming a detoxicating action and others an increase in toxicity. Leo Loeb (1) in 1905 reported experiments showing serum to possess the power of reducing the coagulative activity of tissue extracts. He found that previously heating the serum to 56°C. or 80°C. destroyed this action. Freund (2) in 1909 showed that the toxicity of placental press fluid was destroyed by digesting with human serum at 37°C. for one hour. That is, a toxic dose for a rabbit intravenously became non-toxic after such digestion. Dold (3) made a similar observation in regard to saline extracts of various organs. He found that, while digestion for one hour at 37°C. with fresh homologous serum detoxicated organ extracts, digestion for a similar period with serum heated to 60°C. for one hour was without influence on the toxicity of the extracts. A number of investigators (4), (5), (6), (7), (8), (9), (10) reported findings similar to those of Dold. Gley noted that digestion with serum for only 30 minutes reduced the

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toxicity only slightly, while digestion for one hour reduced it markedly. Roger found that the entire toxicity was present immediately after mixing and that detoxication occurred only after keeping the mixture at 38°C. for one hour. Giafami reduced the toxicity of placental extracts for rabbits by digestion with serum, especially serum of pregnant women. Fetal serum and horse serum he found to be much less efficacious. Schenck also worked with placental press fluid and obtained detoxication even by 15 to 30 minutes' digestion with normal serum.

There have been found results contrary to those mentioned above, however; Ascoli and Izar (11) contended that non-toxic doses of organ extracts could be rendered toxic by digestion with serum for 30 minutes at 37°C. and then keeping for 12 hours at room temperature. Heating the serum to 56°C. for 30 minutes previously they found to prevent this development of increased toxicity, as did also Berkefeld filtration of the tissue extract. Dold and Ogata (12) in repeating their previous work were unable to agree with Ascoli and Izar, but adhered to Dold's previous findings. Cesa-Bianchi (13) was also unable to decrease the toxicity of organ extracts by serum digestion or other biological procedures. He also found, instead, a slight increase in toxicity when digesting with heterologous serum.

From a study of our results set forth in the following pages it will be apparent why different findings may be had, the effect of serum depending greatly on the time elapsing before testing the mixture. Loeb also stated that the effect of the serum was a function of the time and temperature, but he found only a detoxicating action with no tendency toward increased toxicity at the start.

Experimental. The determination of the coagulative activity of the extracts was made in all cases by use of citrated horse plasma. Horse blood was drawn into vessels containing sufficient potassium citrate to make a final concentration of 0.5 per cent, the corpuscles allowed to sediment in an ice box and the clear plasma drawn off. Tissue extracts used were made by grinding the *fresh tissue* (*lung*) with 0.9 per cent NaCl solution for 5 to 10 minutes, filtering through several layers of cheese cloth and sedimenting over night at 3°C. No definite proportions of tissue and saline solution were adhered to, so that the initial activity of extracts varied considerably. Usually the extracts were approximately saturated with the soluble tissue materials, that is, they contained about 2 per cent of coagulable proteins, four-fifths of which was tissue fibrinogen.

The samples of rabbit serum tested were obtained by drawing the blood directly from the rabbit's heart without the use of anesthetic, and, after clotting, permitting separation of the serum by standing over night in an ice box at about 3°C. The tests recorded in table 1 were carried out within 3 days after bleeding the animals.

In making the coagulation tests the normal clotting time for the citrated horse plasma was taken as the time when 1 cc. of it would clot at 41°C. after adding the optimum amount of $CaCl_2$, which was 0.3 cc. of a 0.1 per cent solution. In testing the thromboplastic action of lung extract or serum, 0.1 cc. was added to the citrated plasma and the mixture brought to 41°C. before adding the $CaCl_2$. Clotting time was always measured from the time of adding the $CaCl_2$ and agitating. In testing the lung extract and serum mixtures, equal amounts of the two were mixed, shaken well by hand and placed in the water bath in which the coagulation tests were being conducted. At intervals, as noted in table 1, 0.2 cc. of the mixture was added to the citrated plasma, followed by the proper amount of $CaCl_2$ and the clotting time taken. The amount of the mixture used for the test (0.2 cc.) was so taken in order to have present the same amounts of lung extract and serum as were used in the controls. The principle used in testing the anticoagulant-serum mixtures was identical with that just described for the lung extract-serum mixtures.

Table 1 shows the typical action of rabbit serum on rabbit lung extract.

Rabbit 1 was a normal rabbit from whose heart were drawn 20 cc. of blood for obtaining normal serum.

Rabbit 2 had received two intravenous injections of tissue anticoagulant of 6 cc. each at 6-day intervals, and then ten 1 cc. injections at 1 day intervals, the blood sample for serum being taken 5 days following the last injection. We were trying for a serum here that would destroy either the anticoagulant or active coagulant. It is seen to act just the same as serum from the normal control.

Rabbit 3 received twelve 1 cc. injections of this same anticoagulant solution at intervals of 24 hours, a blood sample being drawn 2 days after the last injection. This sample of serum is labelled (3_1). Ten further daily injections of 1 cc. were given, followed in 5 days by the drawing of a second blood sample (3_2).

Rabbit 4 received the same series of injections as did rabbit 3, but a blood sample was drawn only at the end of the series, comparable to sample (3_2).

Rabbit 5 received two 6 cc. injections of anticoaguiant at 6-day intervals, and was bled 10 days after the second injection.

A study of the results recorded in table 1 will show a variety of interesting facts. *First,* in each case except one (the last one recorded)

the rabbit serum alone showed a thromboplastic action on the clotting of citrated horse plasma by calcium. Thus clotting times of 1 minute 4 seconds, 1 minute 55 seconds, 1 minute 25 seconds and 1 minute 15 seconds were obtained as contrasted with the normal time of 2 minutes 55 seconds.

TABLE 1

CITRATED HORSE PLASMA	1 PER CENT $CaCl_2$	LUNG EXTRACT (RABBIT)	ANTICOAGULANT (HORSE)	SERUM OF RABBIT	COAGULATION TIME OF CONTROLS	COAGULATION TIME AFTER INCUBATING SERUM AND LUNG EXTRACT OR ANTICOAGULANT AT 41°C. FOR:					
						1 minute	5 minutes	10 minutes	30 minutes	1 hour	2 hours
cc.	*cc.*	*cc.*	*cc.*	*cc.*							
1	0.3				2′55″						
1	0.3	0.1			17″						
1	0.3			0.1 (1)	1′12″						
1	0.3	0.1		0.1 (1)		4″	9″	9″	16″	23″	30″
1	0.3		0.1		10′46″						
1	0.3		0.1	0.1 (1)		1′55″	1′55″	1′53″	1′59″	1′55″	
1	0.3			0.1 (2)	1′04″						
1	0.3	0.1		0.1 (2)		5″	7″	7″	10″	11″	
1	0.3		0.1	0.1 (2)		2′00″	1′40″	1′35″	1′28″	1′25″	
1	0.3			0.1 (3_1)	1′55″						
1	0.3	0.1		0.1 (3_1)		8″	15″	30″	50″	58″	
1	0.3		0.1	0.1 (3_1)		2′00″	1′55″	1′55″	1′50″	1′55″	
1	0.3			0.1 (3_2)	1′25″						
1	0.3	0.1		0.1 (3_2)		7″	12″	12″	14″	16″	
1	0.3		0.1	0.1 (3_2)		2′05″	2′05″	2′05″	1′45″		
1	0.3			0.1 (4)	1′15″						
1	0.3	0.1		0.1 (4)		4″	4″	9″	14″	26″	
1	0.3		0.1	0.1 (4)		2′26″	2′35″	2′10″	2′10″	2′05″	
1	0.3			0.1 (5)	4′05″						
1	0.3	0.1		0.1 (5)		10″	11″	12″	17″		
1	0.3		0.1	0.1 (5)		9′00″					

The serum of rabbit 5, on the other hand, was slightly antithrombic the clotting time being 4 minutes 5 seconds, or 1 minute 10 seconds beyond the normal control. *Second*, each of these sera was capable of very markedly increasing the activity of the lung extract, serum 5, which alone was slightly antithrombic, exhibiting this activating power to

a less degree than the rest. The very marked increase in thromboplastic power of the lung extract becomes even more striking when we take into consideration the slight effect that dilution has on this power. Thus in a previous paper (14) Mills has shown that in diluting lung extract with 0.9 per cent NaCl solution, as the concentration of the active material is reduced from 1 to $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, etc., the coagulative efficiency decreases as represented by coagulation times of 10 seconds, 12 seconds, 14 seconds, 17 seconds, 20 seconds, 25 seconds, 30 seconds, etc. Therefore, we see that an increase of activity by the serum causing the lung extract to bring on coagulation in 10 seconds instead of 17 seconds means an eight-fold increase in activity while a clotting time of 8 seconds would mean at least a sixteen-fold increase, and down to 4 seconds would mean more than a thirty-fold increase in the coagulative activity of the lung extract. This tremendous activation occurs very quickly, for it is in evidence even after only about 20 seconds of shaking the serum and lung extract together, even without incubation. It is possibly just this property of rabbit blood that accounts for the very great ease with which intravascular clotting may be induced in rabbits.

The third point of interest in table 1 lies in the results showing the influence of these sera upon our anticoagulant. The anticoagulant used here was a 0.9 per cent NaCl extract of horse lung which had been previously dried at room temperature and thoroughly extracted with benzene, also at room temperature. Such an extract is here seen to have lengthened the clotting time of the horse plasma from 2 minutes 55 seconds up to 10 minutes 46 seconds when used in the ratio of 0.1 cc. for 1 cc. of plasma. The influence of rabbit serum on this anticoagulant solution is seen to be an immediate neutralization, the entire antithrombic action being lost while a part of the thromboplastic action of the serum is also destroyed. The notable exception to this was serum 5, which itself was slightly antithrombic. Here there was very slight reduction in the anticoagulative action of the extract. In no case was there evident an increase in antithrombic activity from the action of the serum, similar to the activation of the lung extract previously noted. There was noticed with serum 2 and serum 4 a slight tendency toward increased thromboplastic activity as the serum and anticoagulant were incubated together.

We see, then, that rabbit serum may very markedly increase the coagulative activity of lung extract (the increase being as high as perhaps thirty-fold) although the serum itself possesses only about one-hundredth

TABLE 2

CITRATED HORSE PLASMA	1 PER CENT $CaCl_2$	LUNG EXTRACT (CALF)	HUMAN SERUM	COAGULATION TIME OF CONTROLS	COAGULATION TIME AFTER INCUBATING SERUM AND LUNG EXTRACT TOGETHER AT 31°C. FOR:							
					1 minute	5 minutes	10 minutes	30 minutes	1 hour	4 hours	6 hours	In ice box 30 hours
cc.	*cc.*	*cc.*	*cc.*									
1	0.3			2′40″								
1	0.3	0.05		14″								
1	0.3		0.05 (1)	2′20″								
1	0.3	0.05	0.05 (1)		10″	10″	12″	12″	13″	15″	14″	20″
1	0.3		0.05 (2)	2′20″								
1	0.3	0.05	0.05 (2)		10″	10″	13″	13″	14″	16″	15″	20″

TABLE 3

CITRATED PLASMA (HORSE)	1 PER CENT $CaCl_2$	LUNG EXTRACT (HORSE)	SYPHILITIC SERUM	COAGULATION TIME OF CONTROLS	COAGULATION TIME AFTER INCUBATING SERUM AND LUNG EXTRACT AT 34°C. FOR:					
					1 minute	5 minutes	10 minutes	30 minutes	1 hour	5 hours
cc.	*cc.*	*cc.*								
1	0.35			2′00″						
1	0.35	0.05		12″						
1	0.35		0.05 cc. Serum I	1′20″						
1	0.35	0.05	0.05 cc. Serum I		9″	10″	10″	11″	13″	14″
1	0.35		0.05 cc. Serum II	1′25″						
1	0.35	0.05	0.05 cc. Serum II		8″	9″	10″	11″	12″	14″
1	0.35		0.05 cc. Serum III	1′40″						
1	0.35	0.05	0.05 cc. Serum III		8″	9″	10″	12″	14″	15″
1	0.35		0.05 cc. Serum IV	1′35″						
1	0.35	0.05	0.05 cc. Serum IV		8″	10″	11″	13″	15″	16″
1	0.35			2′00″						

the thromboplastic activity of the original lung extract. It is very evident that the increased activity is not due merely to an addition of the effects of the two separately, for serum 5, possessing no thromboplastic action whatever, still causes an eight-fold increase in the activity of the lung extract. As yet we have no evidence as to the nature of this activation.

As a natural sequence to our findings in regard to this remarkable property of rabbit serum, we next examined human serum to see if it possessed this same property and to the same degree. Blood samples were obtained from two apparently normal individuals, and, after standing over night in the ice box, the serum was drawn off and tested. The results are shown in table 2.

Here again is noted the same serum effect, only to a milder degree than that obtained with rabbit serum. The increase in coagulative activity here is approximately four-fold. The later inhibitory phase of the serum action is slight in these cases and is later in developing than with the rabbit serum.

Hirschfeld and Klinger (15) in 1914 reported a coagulation reaction which they thought to be specific of syphilitic serum. Fränkel and Thiele (16) obtained similar results. The basis of their test depended on the inactivation of tissue extracts by syphilitic serum, the extracts losing their ability to quicken the clotting time of mixtures of normal serum and citrated plasma. In order to see if syphilitic serum would differ from normal serum by our method of testing, four samples of human serum showing 4 + Wassermann reactions were tested for their influence on lung extract, with results as set forth in table 3.

We see here that syphilitic serum reacts in all ways similar to normal human serum, and does not possess any marked power to destroy the coagulative activity of tissue extracts.

DISCUSSION

We have no theory at present to offer in explaining the remarkable property of rabbit serum (and to a less degree, human serum also) described in the preceding pages. We are offering our findings at the present time for the sake of any practical value they may have in their bearing on the question of intravenous injections of protein solutions in general, and especially organ extracts and serum.

There is a possibility that many cases of sudden reaction following serum injections have been caused by the presence of small amounts of tissue fibrinogen (thromboplastin) in the serum, the blood of the pa-

tient serving to activate the coagulant to such a degree as to make it effective. It takes relatively a much smaller amount of tissue extract to produce almost instantaneous intravascular clotting in rabbits than it does to produce maximal acceleration of the clotting of the same quantity of blood *in vitro*. Thus 0.03 cc. of a saturated lung extract injected intravenously into a rabbit causes clotting in 15 to 20 seconds, but to clot 50 to 75 cc. of citrated plasma *in vitro* at 37°C. with this speed at least 1 cc. of the same extract would be required.

In observing the influence of time on the action of the serum, we see a very possible reason for the conflicting reports of past investigators. Many had noticed that the primary toxicity of tissue extracts remained for 30 minutes after mixing with the serum, but was decreased after 1 hour. We see that this agrees well with several of our findings.

No one, however, had noted the enormous increase in activity immediately after mixing, and it is just this phase of the matter that is of greatest importance in practical medicine.

SUMMARY OF RESULTS

1. Rabbit serum has been found to be capable of causing as high as a thirty-fold increase in the coagulative activity of lung extract. This effect is gradually replaced by a diminution in the activity of the extract below the original as the mixture is left standing.

2. Normal human serum possesses this same power, but to a less degree, both as regards the primary activation and the later inactivation. Syphilitic serum acts exactly as does the normal serum.

3. The practical importance of this activation of tissue extracts lies in its relation to the intravenous injection of protein solutions, such as organ extracts or blood serum, which may be harmless alone but may be activated sufficiently by the patient's blood to become exceedingly toxic.

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